

**Chemical constituents of polyphenols from
Dragon's blood and flavored tea**

By

Qian Hao

Natural Product Chemistry

Graduate School of Biomedical Sciences

(Pharmaceutical Sciences)

Nagasaki University

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Abbreviations

$^1\text{H-NMR}$	Proton nuclear magnetic resonance
$^1\text{H-}^1\text{H COSY}$	Proton- proton homonuclear correlation spectroscopy
$^{13}\text{C-NMR}$	Carbon-13 nuclear magnetic resonance
AcOEt	Ethyl acetate
CC	Column chromatography
DMSO	Dimethyl sulfoxide
EI-MS	Electron ionization mass spectrum
ESI-MS	Electron spray ionization mass spectrum
FAB-MS	Fast atom bombardment mass spectrometry
Fr.	Fraction
HMBC	^1H -Detected heteronuclear multiple bond correlation
HPLC	High performance liquid chromatography
HPLC-DAD	High performance liquid chromatography-diode array detection
HR-FAB-MS	High resolution fast atom bombardment mass spectrometry
HSQC	^1H -Detected heteronuclear single quantum coherence
IR	Infrared
MeOH	Methanol
MS	Mass spectrum
NOE	Nuclear Overhauser effect
NOESY	Nuclear Overhauser effect spectroscopy
TFA	Trifluoroacetic acid
TLC	Thin-layer chromatography
UV	Ultraviolet
UV-Vis	Ultraviolet and visible

1 Introduction

In 2004, “*Guidance for Industry: Botanical Drug Products*” was issued by Food and Drug Administration (FDA), which regulates and supports application of botanical products for new drugs in USA. However, FDA has so far approved only two cases of botanical drugs, Veregen and Fulyzaq.

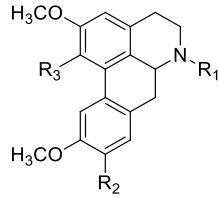
Veregen (Sinecatechins) is an ointment for genital warts caused by infection of a human papillomavirus and contains catechins extracted from green tea and other components. It was the first botanical prescription drug approved by the FDA, in 2006.¹ Fulyzaq (Crofelemer) is red latex from *Croton lechleri* (named Dragon’s blood) in South America for treatment of diarrhea associated with anti-HIV drugs. It was the second case of botanical drug, but the first oral botanical drug approved by FDA, in 2012. The latex is composed of a mixture of polymeric proanthocyanidins.² This example showed that now the study of botanical drug is no longer just interested in monomeric low-molecular-weight compounds.

Dragon’s blood is a common name of a red resin of different genera such as *Croton* sp. (Euphorbiaceae), *Pterocarpus* sp. (Leguminosae), *Daemonorops* sp. (Arecaceae) and *Dracaena* sp. (Agavaceae).³ As a famous traditional medicine, dragon’s blood has been used for the treatment of traumatic and visceral hemorrhages for a long time. In China, dragon’s blood has been imported and used traditionally as an important traditional Chinese medicinal herb for the treatment of traumatic and visceral hemorrhages since Tang dynasty (AD 618–907).⁴ Until 1970s, the red resin of *Dracaena cochinchinensis* S. C. Chen (Agavaceae) found in the southwest of China has been used widely as the substitute for the traditionally imported dragon’s blood.⁵ Chemical constituents of dragon’s blood derived from *Croton* sp., *Pterocarpus* sp., *Daemonorops* sp. and *Dracaena* sp. (Agavaceae) were reported as follows:

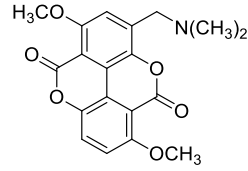
Dragon’s blood produced from the bark of *Croton* sp. (Euphorbiaceae) contains alkaloids, polyphenols, terpenoids and other compounds shown in Chart 1. Among the constituents, oligomeric and polymeric proanthocyanidins are considered to be active

principles of abovementioned Fulyzaq (Crofelemer), which belongs to dragon's blood of this group.

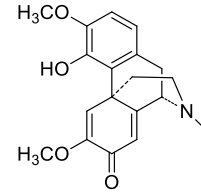
Alkaloids



glaucine **I** R₁=CH₃ R₂=OCH₃ R₃=OCH₃
 isoboldine **II** R₁=CH₃ R₂=OH R₃=OH
 thaliporphine **III** R₁=CH₃ R₂=OCH₃ R₃=OH
 norisoboldine **IV** R₁=H R₂=OH R₃=OH

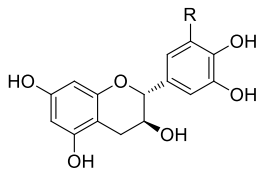


taspine **V**

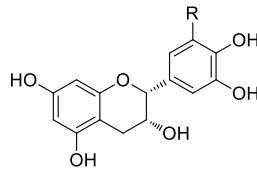


sinoacutine **VI**

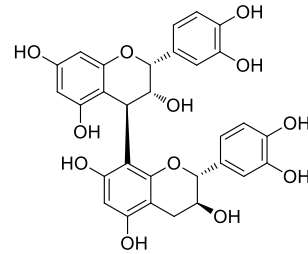
Polyphenols



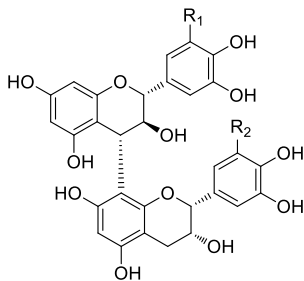
(+)-catechin **VII** R=H
 (+)-gallocatechin **VIII** R=OH



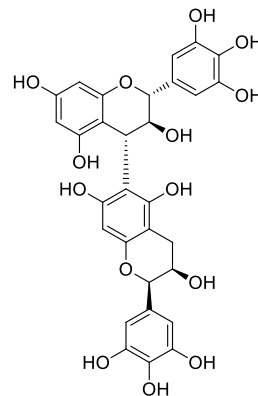
(-)-epicatechin **IX** R=H
 (-)-epigallocatechin **X** R=OH



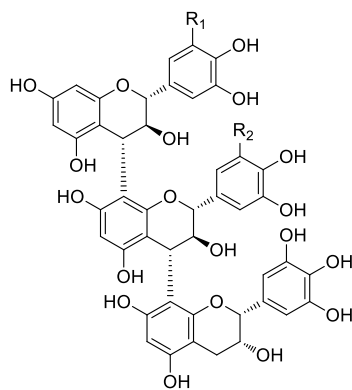
procyanidin B-1 **XI**



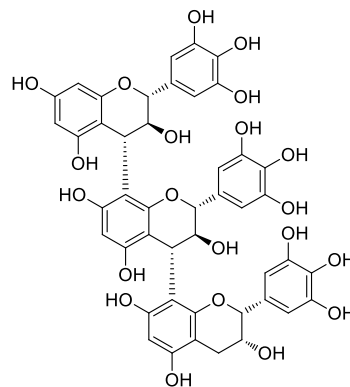
procyanidin B-4 **XII** R₁=H R₂=H
 catechin-(4 α →8)-epigallocatechin **XIII** R₁=H R₂=OH
 gallocatechin-(4 α →8)-epicatechin **XIV** R₁=OH R₂=H



gallocatechin-(4 α →6)-epigallocatechin **XV**

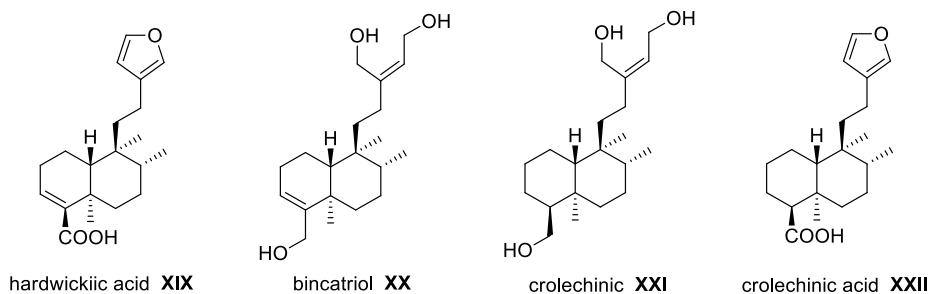


catechin-(4 α →8)-gallocatechin-(4 α →8)-epigallocatechin **XVI** R₁=H R₂=OH
 gallocatechin-(4 α →8)-catechin-(4 α →8)-epigallocatechin **XVII** R₁=OH R₂=H



gallocatechin-(4 α →8)-gallocatechin-(4 α →8)-epigallocatechin **XVIII**

Terpenoids



Others

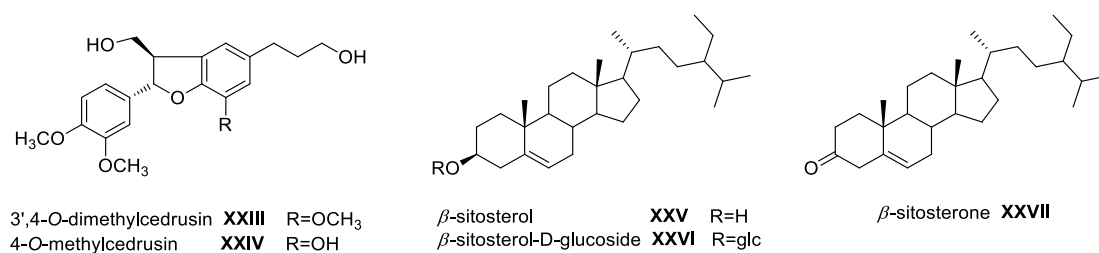
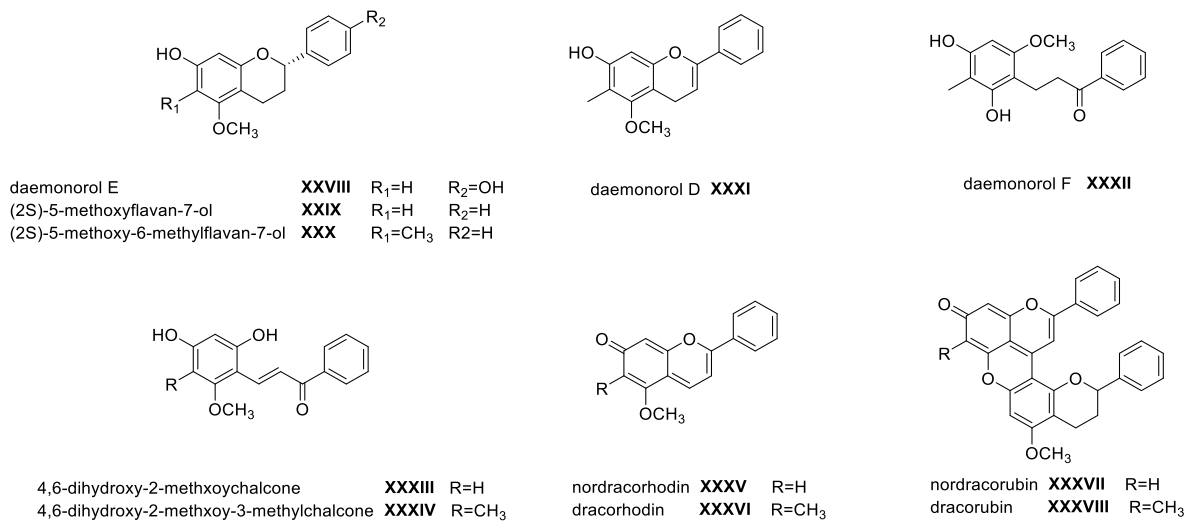


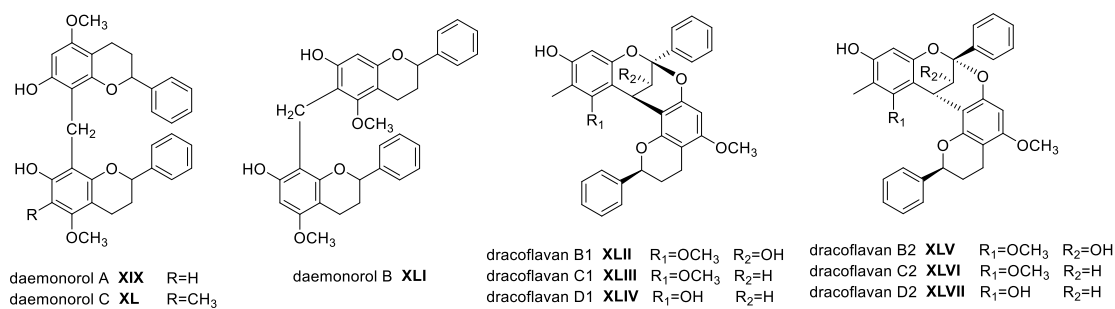
Chart 1. Structures of important constituents of dragon's blood from *Croton* sp.

No major chemical studies have been done on the dragon's blood produced from *Pterocarpus* sp. (Leguminosae), although the plants of this genus contain flavonoids.

Chemical studies on dragon's blood produced from *Daemonorops* sp. (Arecaceae) showed it contains polyphenols and terpenoids (Chart 2).

Polyphenols





Terpenoids

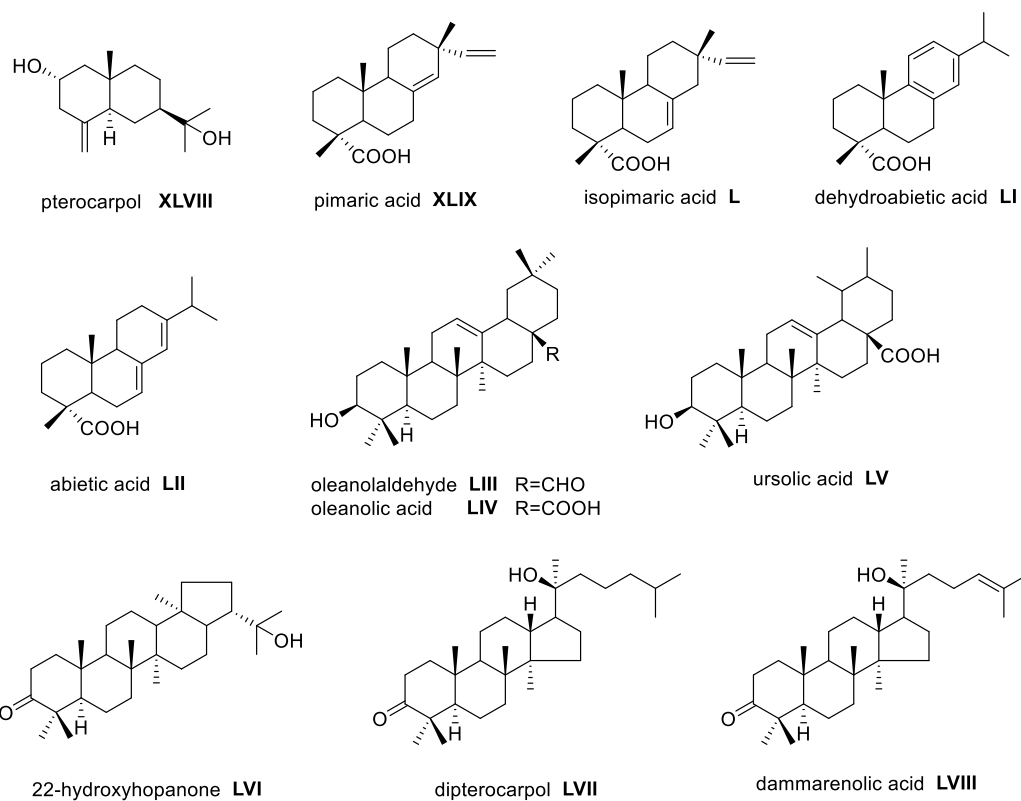
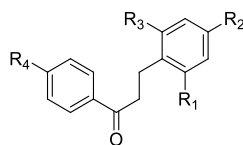


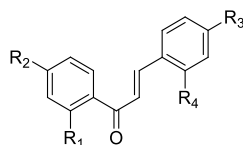
Chart 2. Structures of constituents isolated from the dragon's blood obtained from *Daemonorops* sp.

From Chinese dragon's blood produced from *Dracaena* sp. (Agavaceae), many flavonoids, steroids, saponins and phenolic compounds have so far been isolated (Chart 3).

Chalcones

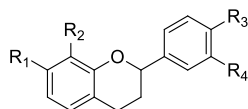


loureirin A	LIX	R ₁ =OCH ₃	R ₂ =OCH ₃	R ₃ =H	R ₄ =OH
loureirin B	LX	R ₁ =OCH ₃	R ₂ =OCH ₃	R ₃ =OCH ₃	R ₄ =OH
loureirin C	LXI	R ₁ =OCH ₃	R ₂ =OH	R ₃ =H	R ₄ =OH
loureirin D	LXII	R ₁ =OCH ₃	R ₂ =OH	R ₃ =OH	R ₄ =OH
cochininenin A	LXIII	R ₁ =OCH ₃	R ₂ =H	R ₃ =OCH ₃	R ₄ =OH
2,4,4'-trihydroxydihydrochalcone	LXIV	R ₁ =OH	R ₂ =OH	R ₃ =H	R ₄ =OH
2-methoxy-4-hydroxydihydrochalcone	LXV	R ₁ =OCH ₃	R ₂ =OH	R ₃ =H	R ₄ =H
4,4'-dihydroxy-2,6-dimethoxydihydrochalcone	LXVI	R ₁ =OCH ₃	R ₂ =OH	R ₃ =OCH ₃	R ₄ =OH
2,4'-dihydroxy-4,6-dimethoxydihydrochalcone	LXVII	R ₁ =OCH ₃	R ₂ =OCH ₃	R ₃ =OH	R ₄ =OH

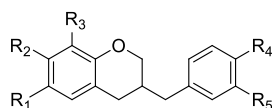


2,4,4'-trihydroxychalcone	LXVIII	R ₁ =OH	R ₂ =OH	R ₃ =OH	R ₄ =H
2'-methoxy-4,4'-trihydroxychalcone	LXIX	R ₁ =H	R ₂ =OH	R ₃ =OH	R ₄ =OCH ₃
2-methoxy-4,4'-trihydroxychalcone	LXX	R ₁ =OCH ₃	R ₂ =OH	R ₃ =OH	R ₄ =H

Flavanes

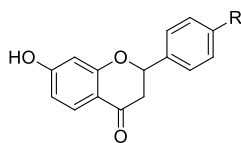


4',7-dihydroxyflavan	LXXI	R ₁ =OH	R ₂ =H	R ₃ =OH	R ₄ =H
7-hydroxy-4'-methoxyflavan	LXXII	R ₁ =OH	R ₂ =H	R ₃ =OCH ₃	R ₄ =H
3',7-dimethoxy-4'-hydroxyflavan	LXXIII	R ₁ =OCH ₃	R ₂ =H	R ₃ =OH	R ₄ =OCH ₃
4',7-dihydroxy-3'-methoxyflavan	LXXIV	R ₁ =OH	R ₂ =H	R ₃ =OCH ₃	R ₄ =OH
4',7-dihydroxy-8-methylflavan	LXXV	R ₁ =OH	R ₂ =CH ₃	R ₃ =OH	R ₄ =H
4',7-dihydroxy-8-methyl-3'-methoxyflavan	LXXVI	R ₁ =OH	R ₂ =CH ₃	R ₃ =OH	R ₄ =OCH ₃

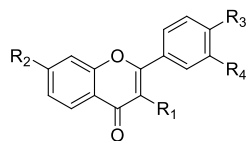


7-hydroxy-(4'-hydroxybhenyl) chromane	LXXVII	R ₁ =H	R ₂ =OH	R ₃ =H	R ₄ =OH	R ₅ =H
6-hydroxy-7-methoxy-(4'-hydroxybhenyl) chromane	LXXVIII	R ₁ =OH	R ₂ =OCH ₃	R ₃ =H	R ₄ =OH	R ₅ =H
7-hydroxy-(3'-hydroxy-4'-methoxybhenyl) chromane	LXXIX	R ₁ =H	R ₂ =OH	R ₃ =H	R ₄ =OCH ₃	R ₅ =OH
7-hydroxy-8-methoxy-(4'-hydroxybhenyl) chromane	LXXX	R ₁ =H	R ₂ =OH	R ₃ =OCH ₃	R ₄ =OH	R ₅ =H

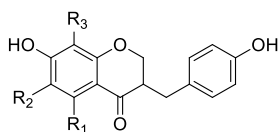
Flavones



7-hydroxy-dihydroflavone **LXXXI** R=H
7,4'-dihydroxy-dihydroflavone **LXXXII** R=OH

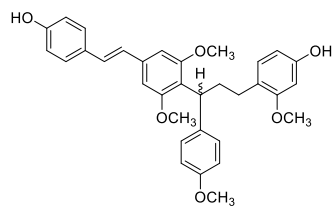


7,4'-dihydroxyflavone **LXXXIII** R₁=H R₂=OH R₃=OH R₄=H
3,4'-dihydroxy-7-methoxyflavone **LXXXIV** R₁=OH R₂=OCH₃ R₃=OH R₄=H
4'-methoxy-3',7-dihydroxyflavone **LXXXV** R₁=H R₂=OH R₃=OCH₃ R₄=OH

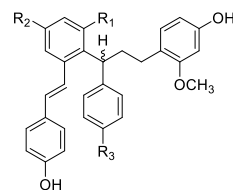


4',7-dihydroxy-5-methoxyhomoisoflavone **LXXXVI** R₁=OCH₃ R₂=H R₃=H
4',7-dihydroxy-8-methylhomoisoflavone **LXXXVII** R₁=H R₂=H R₃=CH₃
4',5,7-trihydroxyhomoisoflavone **LXXXVIII** R₁=OH R₂=H R₃=H
4',7-dihydroxyhomoisoflavone **LXXXIX** R₁=H R₂=H R₃=H
4',5,7-trihydroxy-6-methylhomoisoflavone **XC** R₁=OH R₂=CH₃ R₃=H

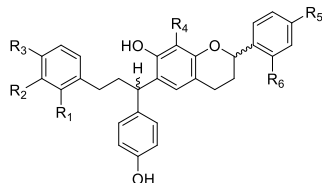
Flavonoid oligomers



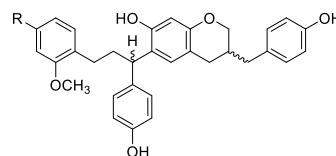
cochinchenene A **XCI**



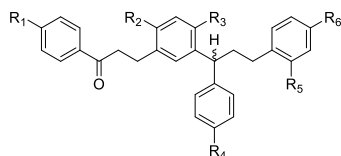
cochinchenene B **XCII** R₁=OH R₂=OCH₃ R₃=OCH₃
cochinchenene C **XCIII** R₁=OCH₃ R₂=OH R₃=OH
cochinchenene D **XCIV** R₁=OH R₂=OH R₃=OH



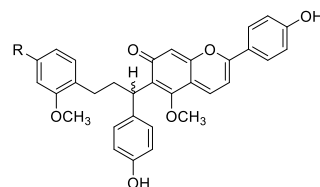
8-methylsocotrin-4'-ol **XCV** R₁=OCH₃ R₂=H R₃=OH R₄=CH₃ R₅=OH R₆=H
socotrin-4'-ol **XCVI** R₁=OCH₃ R₂=H R₃=OH R₄=H R₅=OH R₆=H
cochinchenin D **XCVII** R₁=OH R₂=CH₃ R₃=OCH₃ R₄=H R₅=OCH₃ R₆=OH



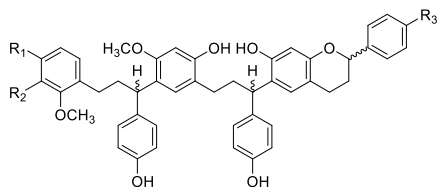
homoisosocotrin-4'-ol **XCVIII** R=OH
cochinchenin E **XCIX** R=OCH₃



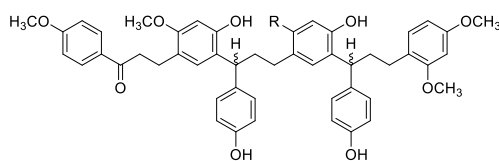
cochinchenin B **C** R₁=OH R₂=OCH₃ R₃=OCH₃ R₄=OH R₅=OCH₃ R₆=OH
cochinchenin C **CI** R₁=OH R₂=OCH₃ R₃=OH R₄=OCH₃ R₅=OCH₃ R₆=OH
cochinchenin F **CIII** R₁=OCH₃ R₂=OH R₃=OCH₃ R₄=OH R₅=OH R₆=OCH₃
cinnabarone **CIII** R₁=OH R₂=OH R₃=OCH₃ R₄=OH R₅=OH R₆=OCH₃



dracaenin A **CIV** R=OH
dracaenin B **CV** R=OCH₃



cochinchenin G **CVI** R₁=OCH₃ R₂=OCH₃ R₃=OH
damalchawin **CVII** R₁=OH R₂=H R₃=H



cochinchenin H **CVIII** R=OCH₃

Others

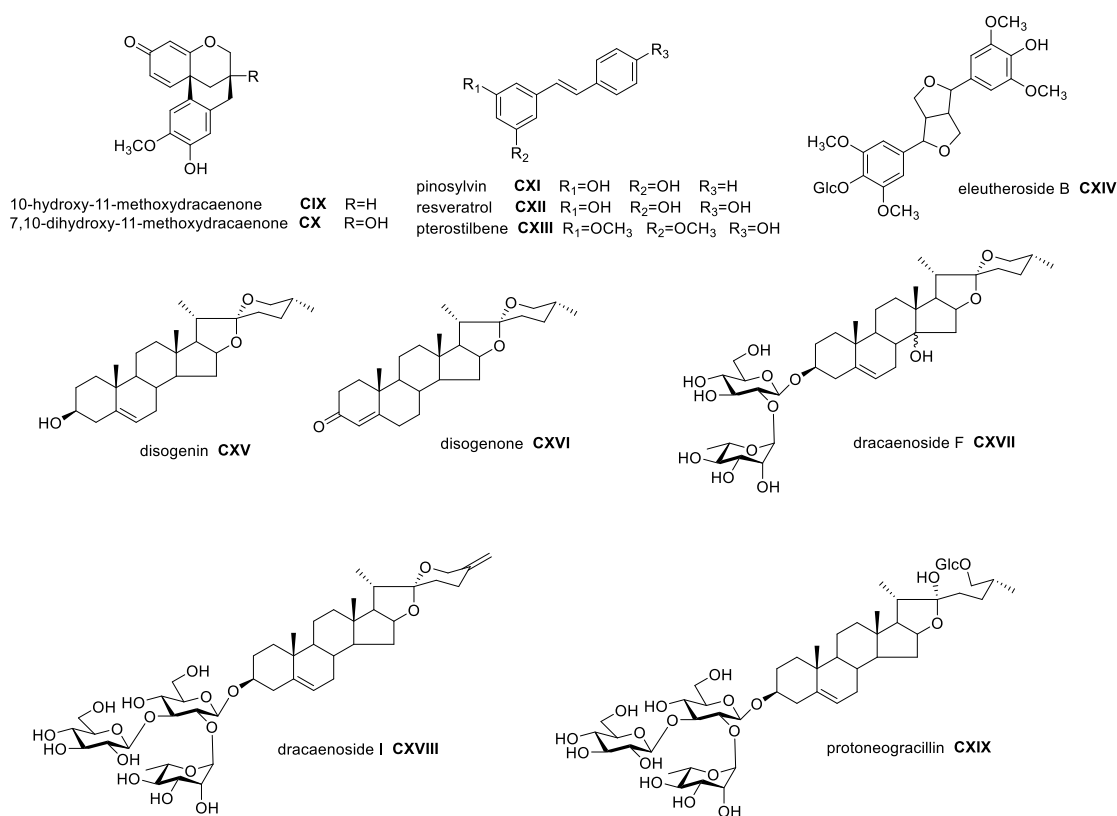


Chart 3. Structures of constituents reported from the dragon's blood produced from *Dracaena* sp.

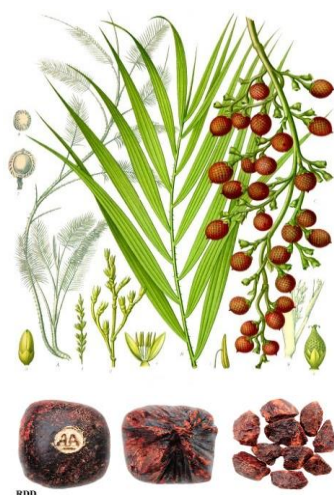


Figure 1. Morphological characteristics of *Daemonorops draco* plant and Dragon's blood medicinal materials.

Dragon's blood resin of *Daemonorops draco* is obtained as deep red teardrop-shaped lumps (Figure 1), which are separated from the immature fruit of *Daemonorops draco* (the Southeast Asian rattan- or cane-palm). The traditional preparation method of the lumps is as follows: the fruits were placed in sacks and ponded. Then the pulp was treated with boiling water to give the resinous layer. Subsequently the resin was kneaded into balls or long sticks³. Previous research on the resin showed that major compounds are flavonoids and terpenoids;⁶⁻⁷ however, only a part of constituents

of the total resin has so far been clarified.

Chinese dragon's blood is the red resin obtained from stems of *Dracaena cochinchinensis*. *D. cochinchinensis* is exclusively distributed in China (southern Yunnan and Guangxi provinces), Myanmar and Laos, and it has been listed as a national endangered plant since 1987.⁸ This plant is generally found on sunny cliffs in limestone areas of steep mountains, typically at elevations of 1,300–1,700 m. Botanists estimate that there are only about 200,000 plants in the wild, and the wild resource is becoming scarce more and more due to overcollection.⁹⁻¹⁰ This red resin is formed gradually with the microbial infection or (and) the natural oxidation after the wounding the wood.¹¹⁻¹² Previous phytochemical studies showed that the resin mainly contains phenolic compounds, including monomeric and oligomeric flavonoids, stilbenoids, and several steroids (Chart 3).¹³ However, the major constituents of this resin, that is uncharacterized polymeric substance, are still unclear.



Figure 2. Morphological characteristics of *Dracaena cochinchinensis* and Dragon's blood produced in the stem xylem.^{10,12}

This thesis described an extended study on uncharacterized polymeric polyphenols generated in flavored tea. Tea (*Camellia sinensis*) is the most popular drink worldwide. Recent biological and epidemiological studies indicated that the tea polyphenols, mainly composed of epigallocatechin and its galloyl esters, possess a broad spectrum of biological activities, including anti-oxidant properties, reduction of various cancers, inhibition of inflammation and protective effects against diabetes, hyperlipidemia and obesity.¹⁴ There are many types of processed tea products in the

world, such as green tea, black tea, oolong tea, post-fermented tea, and flavored tea. Flavored tea originated in China, and produced by mixing tea products with spices, fruits, and flowers. This type of tea was mainly cherished by Chinese and European people and has recently become popular in Japan. Cinnamon tea is a typical flavored tea produced by simple blending of cinnamon bark and black tea, and the study described in Chapter 4 revealed that reaction of the cinnamon essential oil with tea catechins generate characteristic polyphenols.

This thesis consists of five chapters. Chapter 1 gave a general overview of the dragon's blood and flavored tea, as well as the outline of this study. Chapter 2 through Chapter 4 give the details of the individual chemical studies on dragon's blood produced from *Daemonorops draco* and *Dracaena cochinchinensis*, and Cinnamon tea, respectively. Chapter 5 summarizes the results obtained in the studies. The methods and experimental data are given in the Experimental Section, which is finally followed by the reference section, listing the literatures cited in the text.

2 Chemical constituents of dragon's blood from *Daemonorops draco*

2.1 Introduction

Dragon's blood resin, obtained from the fruit of *Daemonorops draco* (Arecaceae), is a precious traditional medicine and also has been used as a colorant in artwork in many cultures since ancient times.¹⁴ *D. draco* distributed mainly in South East Asia, Indonesia and Malaysia. For centuries, people use the resin to stimulate blood circulation, heal wound, alleviate pain, control bleeding, and promote tissue regeneration.^{3,16}

The commercial resin has been previously investigated and presence of red pigments, flavans, C-methylated flavans, chalcones, biflavonoids, triflavonoids, deoxyproanthocyanidins, diterpene acids, and triterpenes were reported.^{6-7,17} Major components are flavonoids and terpenoids, and biological studies suggested that flavonoids are important for the activities. However, preliminary HPLC analysis indicated presence of many uncharacterized phenolic constituents in the commercial resin. Therefore, the aim of this chapter is phytochemical reinvestigation of this resin.

2.2 Extraction and Isolation

Plant Material The resin of *Daemonorops draco* was purchased in Guangdong, China, in 2012, and identified by Prof. Hai-Zhou Li at Kunming University of Science and Technology. A voucher specimen (DD20121001) was deposited in the Laboratory of Natural Product Chemistry, Graduate School of Biomedical Sciences, Nagasaki University, Japan.

D. draco resin (120 g) was thoroughly suspended in CHCl₃ (1.0 L) and stirred at room temperature. The insoluble materials were removed by filtration and the filtrate was concentrated under reduced pressure to yield a CHCl₃-soluble fraction (104.8 g). The extract was subjected to CC on a silica gel column (8 cm i.d. × 25 cm, CHCl₃-MeOH-H₂O 100:0:0–90:10:1–80:20:2–0:100:0) to give four fractions: Fr. 1 (12.2 g), Fr. 2 (11.5 g), Fr. 3 (58.0 g) and Fr. 4 (5.8 g). Fractions 1 and 2 were shown to contain mainly terpenoids by TLC analysis. Fraction 3 was separated on a Sephadex LH-20 column with EtOH and then 60% acetone to give five fractions. Fr. 3-3 was separated on a Diaion HP20SS column with

MeOH-H₂O (40–100%, 20% stepwise elution) to give five fractions. Fr. 3-3-1 and Fr. 3-3-2 were individually separated by a combination of column chromatography over silica gel, Chromatorex ODS column, preparative reverse-phase HPLC and normal-phase HPLC to yield three new compounds along with eight known compounds (Chart 4).

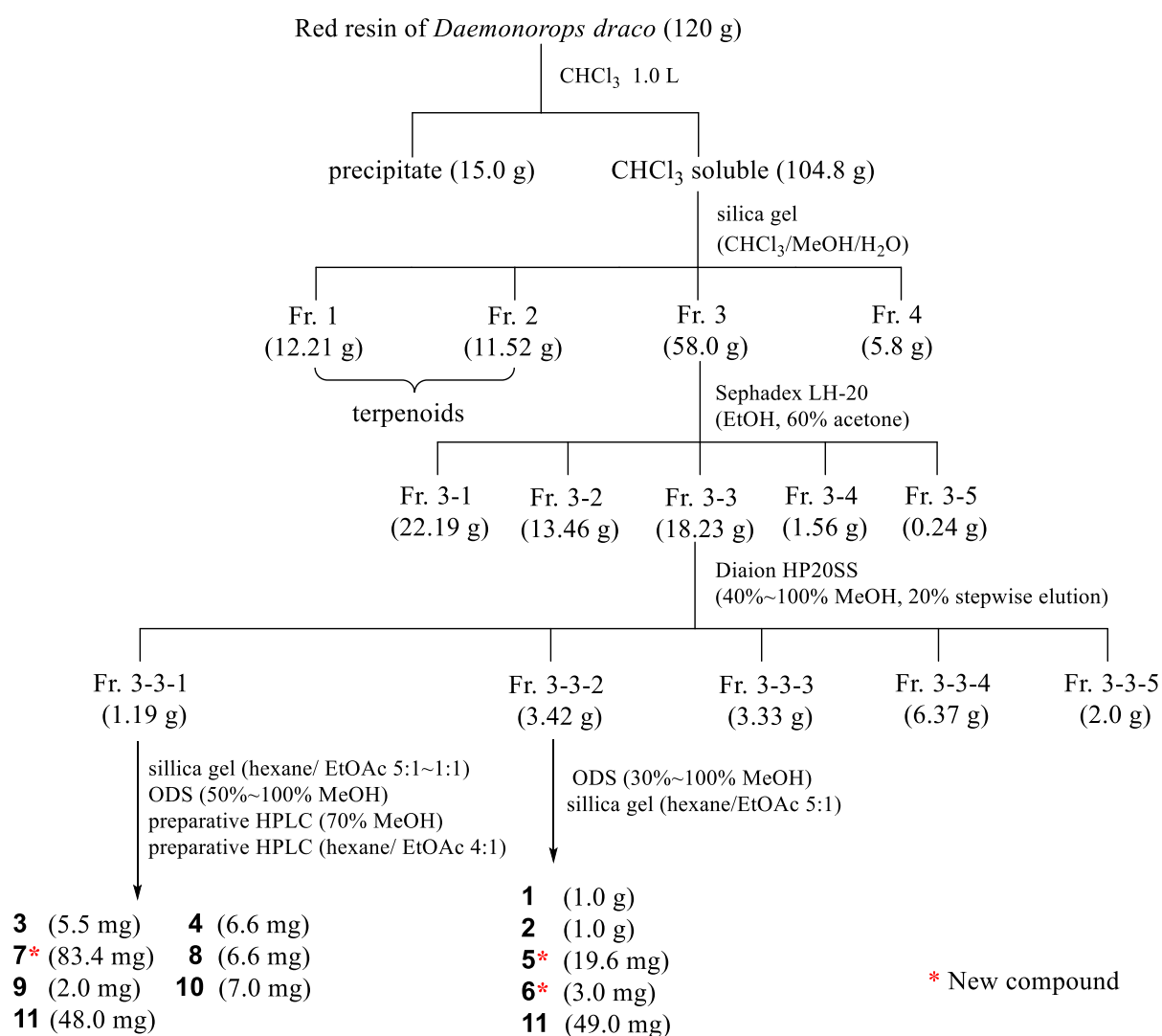


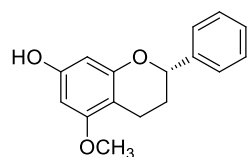
Chart 4. Extraction and separation of the red resin from *D. draco*.

2.3 Structures of known compounds

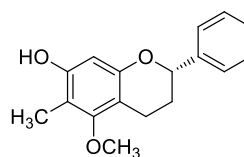
Based on comparison of the spectroscopic data with those found in literatures, the known compounds were identified as four flavans [7-hydroxy-5-methoxyflavan (**1**),⁷

7-hydroxy-5-methoxy-6-methylflavan (**2**),⁷ 7,4'-dihydroxy-8-methylflavan (**3**),¹⁸ and 5,4'-dihydroxy-7-methoxy-6-methylflavan (**4**)¹⁹] two homoisoflavans [7,4'-dihydroxy-8-methoxyhomoisoflavane (**8**)²⁰ and 6,4'-dihydroxy-7-methoxyhomoisoflavane (**9**)²¹] and two chalcones [4,4'-dihydroxy-2,6-dimethoxy-dihydrochalcone (**10**)²² and 4,6-dihydroxy-3-methyl-2-methoxychalcone (**11**)²³].

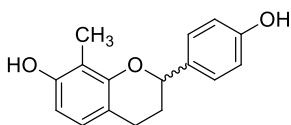
Flavans



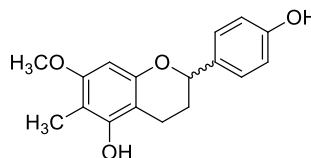
7-hydroxy-5-methoxyflavan (**1**)



7-hydroxy-5-methoxy-6-methylflavan (**2**)

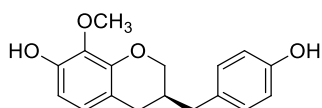


7,4'-dihydroxy-8-methylflavan (**3**)

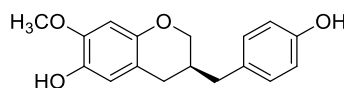


5,4'-dihydroxy-7-methoxy-6-methylflavan (**4**)

Homoisoflavans

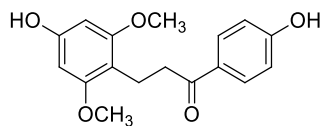


7,4'-dihydroxy-8-methoxyhomoisoflavane (**8**)

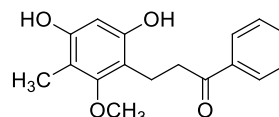


6,4'-dihydroxy-7-methoxyhomoisoflavane (**9**)

Chalcones



4,4'-dihydroxy-2,6-dimethoxydihydrochalcone (**10**)



4,6-dihydroxy-3-methyl-2-methoxychalcone (**11**)

Figure 3. Structures of known compounds isolated from the red resin of *D. draco*.

2.4 Structure elucidation of new compounds

1) 7-Hydroxy-2,5-dimethoxy-6,8-dimethylflavan (**5**)

Compound **5** was obtained as a white amorphous powder and the molecular formula was deduced to be C₁₉H₂₂O₄ on the basis of high-resolution electron impact mass spectrometry (HR-EI-MS) (*m/z* 314.1508 [M]⁺, calcd for C₁₉H₂₂O₄: 314.1519).

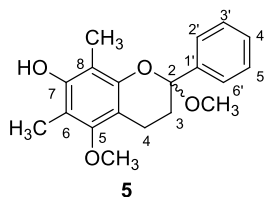


Figure 4. Structure of **5**.

The ¹H NMR spectrum of **5** (Table 1) indicated the presence of one monosubstituted benzene ring [δ_{H} 7.58 (2H, dd, $J = 7.3, 1.4$ Hz), 7.41 (2H, t, $J = 7.3$ Hz), and 7.34 (1H, tt, $J = 7.3, 1.4$ Hz)], and a set of mutually coupled methylene protons [δ_{H} 2.26 (1H, ddd, $J = 13.5, 6.2, 2.3$ Hz), 1.74 (1H, ddd, $J = 13.5, 12.6, 6.2$ Hz), and 2.86 (1H, ddd, $J = 16.0, 12.6, 6.2$ Hz), 2.68 (1H, ddd, $J = 16.0, 6.2, 2.3$ Hz)]. In addition, signals attributable to two methoxy groups [δ_{H} 3.66 (3H, s), and 3.00 (3H, s)], and two methyls [δ_{H} 2.18 (3H, s), and 2.10 (3H, s)] attached to *sp*² carbons were also observed. The ¹³C NMR and HSQC spectra indicated the presence of an acetal carbon (δ_{C} 101.1) and a fully substituted benzene ring (δ_{C} 155.3, 153.4, 149.7, 111.8, 109.9, and 108.8), chemical shifts of which indicated that this aromatic ring has a phloroglucinol-type substitution related to flavonoid A-rings. The connection of the monosubstituted benzene ring to the acetal carbon (δ_{C} 101.1, C-2) was confirmed by the HMBC correlations of H-2', 6' (δ_{H} 7.58) to C-2 (Figure 5). The acetal carbon also showed HMBC cross peaks with the methoxy proton resonance at δ_{H} 3.00 and signals of the methylene protons of C-3. The substitution of the remaining phloroglucinol ring was determined as follows (Figure 5): the methoxy singlet at δ_{H} 3.66 and the C-4 methylene signals showed HMBC correlations to an aromatic carbon signal at δ_{C} 155.3 (C-5), and the C-5 carbon was also correlated with one of the two methyl groups (δ_{H} 2.10) attached to C-6

(δ_C 111.8). The other methyl singlet signal (δ_H 2.18) showed cross peaks with aromatic carbons observed at δ_C 109.9 (C-8), 149.7 (C-8a), and 153.4 (C-7). On the basis of this spectroscopic evidence, the structure of **5** was determined to be 7-hydroxy-2,5-dimethoxy-6,8-dimethylflavan. This compound was concluded to be a mixture of enantiomers, because the specific rotation value, $[\alpha]_D$ 0° ($c=0.14$, MeOH), indicated that this compound is a racemate.

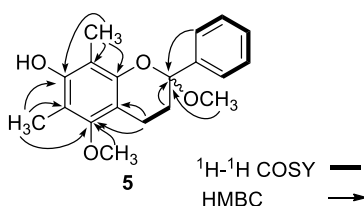


Figure 5. Selected ^1H - ^1H COSY and HMBC correlations of **5**.

2) 7-Hydroxy-2,5-dimethoxy-8-methylflavan (**6**)

Compound **6** was obtained as a white amorphous powder, and its molecular formula was determined to be $\text{C}_{18}\text{H}_{20}\text{O}_4$ by HR-EI-MS analysis based on the $[\text{M}]^+$ peak at m/z 300.1347 (calcd for $\text{C}_{18}\text{H}_{20}\text{O}_4$: 300.1362). The ^1H and ^{13}C NMR spectra of **6** (Table 1) were closely related to those of **5** and showed signals arising from a monosubstituted benzene ring, an acetal carbon (C-2), mutually coupled two methylene groups (C-3 and C-4), and two methoxy groups. The presence of a phloroglucinol ring in the molecule was also indicated by the ^{13}C NMR data; however, the ^1H NMR spectrum of **6** showed an aromatic methyl signal at δ_H 2.11 and an aromatic methine singlet signal at δ_H 6.11. These observations suggested that **6** is a desmethyl analogue of **5**, lacking one aromatic methyl group on the phloroglucinol ring. This was confirmed by examination of the HMBC spectrum of **6** (Figure 6), which showed cross peaks of the aromatic methyl signal (δ_H 2.11) to an aromatic quaternary carbon (δ_C 105.3, C-8) and two oxygen-bearing aromatic carbons (δ_C 155.2, C-7 and δ_C 152.1, C-8a). Another oxygen-bearing aromatic carbon at δ_C 156.8 (C-5) was correlated with methoxy protons (δ_H 3.74), the aromatic methine singlet (H-6), and C-4 methylene protons (Figure 6). Accordingly, compound **6** was characterized as 7-hydroxy-2,5-dimethoxy-8-methylflavan. The specific

rotation, $[\alpha]_D^{24} 0^\circ$ ($c=0.14$, MeOH), suggested that this compound is also obtained in racemic form.

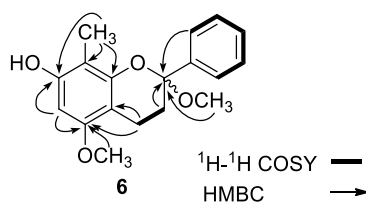


Figure 6. Selected ¹H-¹H COSY and HMBC correlations of **6**.

3) 7-Hydroxy-2,5-dimethoxy-6-methylflavan (**7**)

The molecular formula of compound **7** was shown to be C₁₈H₂₀O₄ (HR-EI-MS: m/z 300.1359 [M]⁺, calcd for C₁₈H₂₀O₄: 300.1362), which is the same as that of **6**. The ¹H and ¹³C NMR spectra of **7** (Table 1) were also closely related to those of **6**, showing signals attributable to mono- and penta-substituted benzene rings, two methylenes, one acetal carbon, and two methoxy groups. In the HMBC spectrum of **7** (Figure 7), the oxygen-bearing C-5 carbon (δ_C 158.1) was correlated with C-4 methylene protons, methoxy protons (δ_H 3.67), and aromatic methyl protons (δ_H 2.06), confirming location of the aromatic methyl group at C-6 position. Consequently, **7** was determined to be 7-hydroxy-2,5-dimethoxy-6-methylflavan.

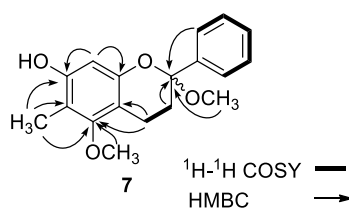


Figure 7. Selected ¹H-¹H COSY and HMBC correlations of **7**.

Table 1. ^1H (500 MHz) and ^{13}C (125 MHz) NMR data for **5-7** (in CD_3OD)^a

position	5		6		7	
	δ_{H}	δ_{C}	δ_{H}	δ_{C}	δ_{H}	δ_{C}
2		101.1		101.2		101.2
3	2.26 (1H, ddd, $J = 13.5, 6.2, 2.3$ Hz) 1.74 (1H, ddd, $J = 13.5, 12.6, 6.2$ Hz)	35.1	2.23 (1H, ddd, $J = 13.5, 6.4, 2.5$ Hz) 1.74 (1H, ddd, $J = 13.5, 12.4, 6.4$ Hz)	34.9	2.21 (1H, ddd, $J = 13.5, 6.2, 2.3$ Hz) 1.72 (1H, ddd, $J = 13.5, 12.6, 6.2$ Hz)	35.4
4	2.86 (1H, ddd, $J = 16.0, 12.6, 6.2$ Hz) 2.68 (1H, ddd, $J = 16.0, 6.2, 2.3$ Hz)	17.7	2.67 (1H, ddd, $J = 16.3, 12.4, 6.4$ Hz) 2.57 (1H, ddd, $J = 16.3, 6.4, 2.5$ Hz)	17.3	2.83 (1H, ddd, $J = 16.0, 12.6, 6.2$ Hz) 2.64 (1H, ddd, $J = 16.0, 6.2, 2.3$ Hz)	17.6
4a		108.8		103.6		108.4
5		155.3		156.8		158.1
6		111.8	6.11 (1H, s)	92.7		111.6
7		153.4		155.2		156.2
8		109.9		105.3	6.29 (1H, s)	100.5
8a		149.7		152.1		152.3
1'		142.9		143.0		142.7
2', 6'	7.58 (2H, dd, $J = 7.3, 1.4$ Hz)	127.3	7.58 (2H, dd, $J = 7.3, 1.4$ Hz)	127.3	7.53 (2H, dd, $J = 7.3, 1.4$ Hz)	127.3
3', 5'	7.41 (2H, t, $J = 7.3$ Hz)	129.4	7.41 (2H, t, $J = 7.3$ Hz)	129.4	7.38 (2H, t, $J = 7.3$ Hz)	129.3
4'	7.34 (1H, tt, $J = 7.3, 1.4$ Hz)	129.2	7.34 (1H, tt, $J = 7.3, 1.4$ Hz)	129.2	7.32 (1H, tt, $J = 7.3, 1.4$ Hz)	129.2
2-OCH ₃	3.00 (3H, s)	50.2	2.99 (3H, s)	50.2	3.02 (3H, s)	50.4
5-OCH ₃	3.66 (3H, s)	60.4	3.74 (3H, s)	55.7	3.67 (3H, s)	60.3
6-CH ₃	2.10 (3H, s)	9.3			2.06 (3H, s)	8.8
8-CH ₃	2.18 (3H, s)	8.6	2.11 (3H, s)	8.0		

^a Chemical shifts are given in δ values, multiplicities and coupling constants (J in Hz) in parentheses.

2.5 HPLC analysis of Dragon's blood produced from *Daemonorops draco*

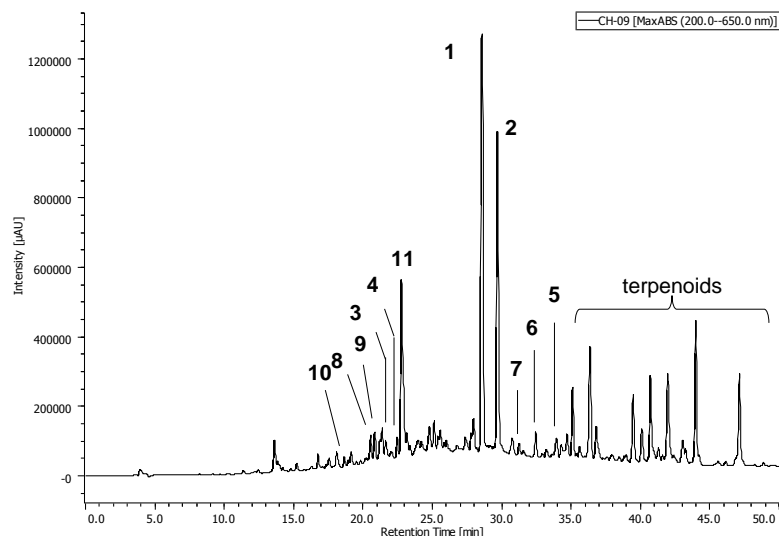


Figure 8. HPLC profile of the red resin from *D. draco* (detection at max UV absorbance)

The HPLC analysis of the red resin from *D. draco* (Figure 8) showed that major constituents of this resin are flavonoids and terpenoids. In the HPLC profile, peaks attributable to 11 phenolic compounds isolated in this study were identified. The major flavonoids were **1**, **2** and **11**, and new compounds **5**, **6** and **7** were minor in this resin.

2.6 Conclusions

In this study, three new flavans (**5-7**), together with eight known flavonoids including flavans, homoisoflavanes, and chalcones, were isolated from the resin of *D. draco*. These new compounds are the first examples of 2-methoxyflavans from *D. draco*. The methoxy groups at the C-2 positions may suggest that new compounds (**5-7**) are artifacts produced during the separation procedure; however, HPLC analysis of the extract prepared by aqueous acetone indicated presence of these compounds in the original resin (Figure 8). The major components were **1** and **2**, followed by **11**. Interestingly, the location of the carbonyl group at the C-2 of **11** is different from that

of usual dihydrochalcones produced by shikimate-acetate pathway, which has the carbonyl group at the position adjacent to the phloroglucinol A ring (Figure 9). This observation suggested a biogenetic relationship between **2**, **11** and **7** (Figure 10): **7a**, an analogue of **7** bearing a hydroxyl group at the C-2 position, is regarded as an oxidation product of **2**, and **7a** is also considered to be produced by intramolecular cyclization of **11**. Although **7a** has not been isolated from this resin, it was strongly suggested that **11** is oxidatively produced by oxidation of **2**, and compound **7** is produced from the unstable intermediate **7a**. Actually, HPLC analysis of a solution of **7** in MeOH showed a peak corresponding to **11**. Thus, our results propose a production mechanism of chalcones in dragon's blood produced from *D. draco*. The anti-platelet effect of flavonoids constituents with various types have been confirmed by many reports in the literature, particularly, it has been reported that the underlying mechanism for anti-platelet activity of **2** was related to inhibition of TXA₂ formation via the inhibition of COX.²⁴⁻²⁵ Based on this finding, we believe that the flavonoids are responsible for the clinical effects of the resin.

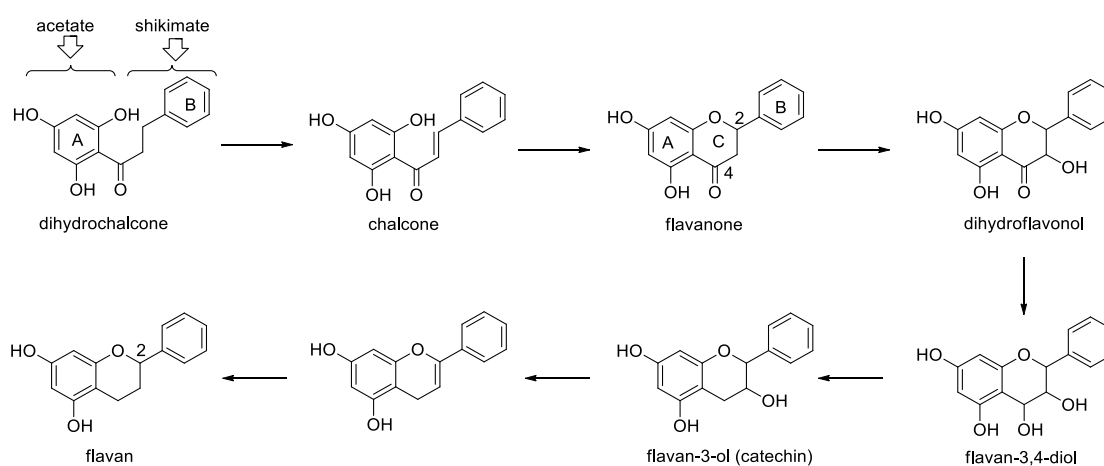


Figure 9. Biogenetic relationship between usual chalcone and flavan.

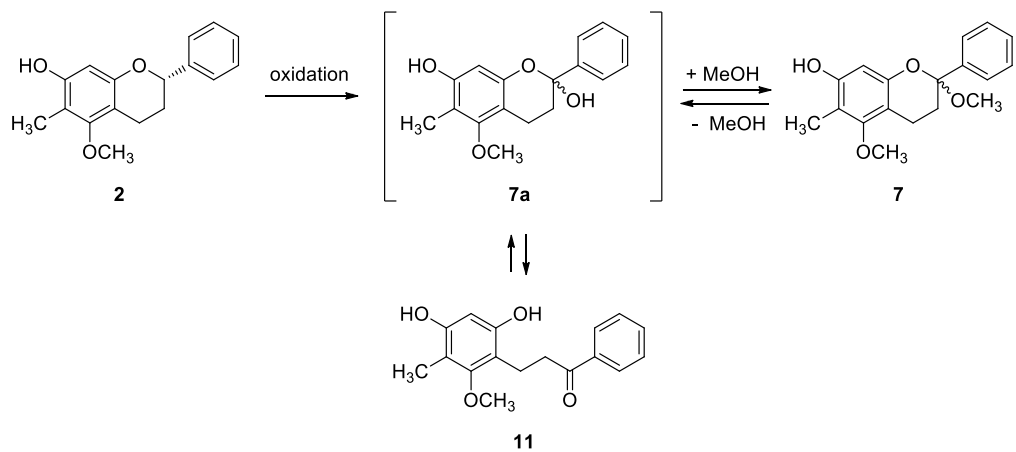


Figure 10. Biogenetic relationship between **2**, **7**, **11** and an intermediate **7a**.

3 Chemical constituents of dragon's blood from *Dracaena cochinchinensis*

3.1 Introduction

Dragon's blood is a name of traditional medicines produced from red resins obtained from different species of four distinct genera and used in many cultures of the world through the ages.²⁶ Chinese dragon's blood is the red resin obtained from stems of *Dracaena cochinchinensis* and has been shown to promote blood circulation, to alleviate inflammation, and to treat stomach ulcers, diarrhea, diabetes, and bleed.²⁷ *D. cochinchinensis* is an evergreen tree or shrub native to the tropical region of Southwest China (Southern Yunnan and Guangxi provinces), Myanmar and Laos, and the resin is produced by artificial wounding of the stem.¹²

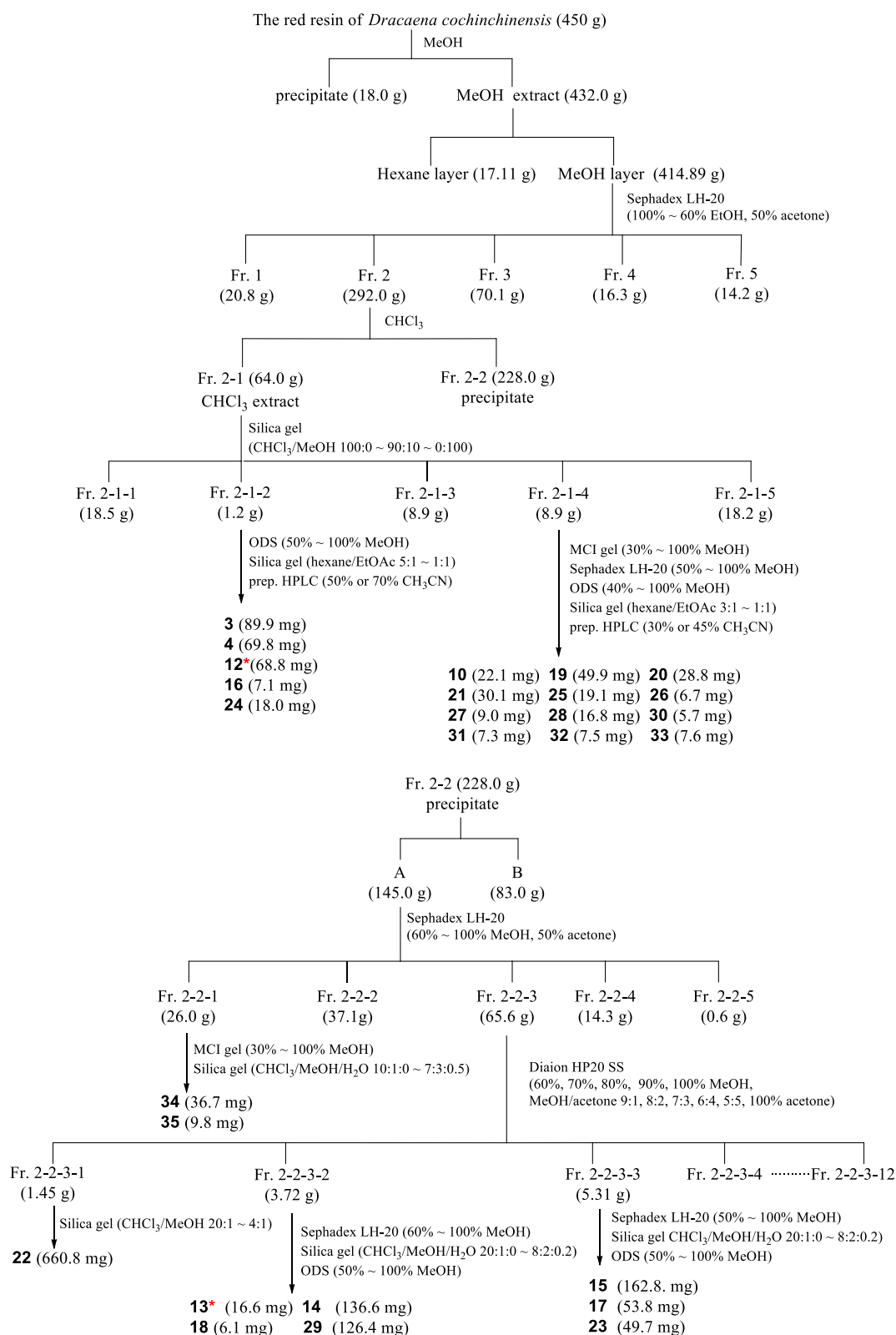
Preceding phytochemical studies showed that flavonoids and steroids are main chemical constituents of the fresh stem of *D. cochinchinensis*, and the resin produced from the stem mainly also contains phenolic compounds, including flavonoids and stilbenoids, as well as several steroids.²⁸⁻²⁹ However, composition of the phenolic substance of the resin is dramatically different from that of fresh plant probably due to enzymatic oxidation with endogenous enzymes and/or metabolism with exogenous microorganisms during production process of the resin.¹²⁻¹³ The major part of the phenolic constituents of the resin are still chemically ambiguous due to the difficulty of separation. Especially, polymeric polyphenols, which increase remarkably after wounding of the plant, remains chemically uncharacterized. The following sections describe the isolation and structure elucidation of the chemical constituents of the resin of *D. cochinchinensis*, which consist of two new phenolic metabolites and 25 known compounds. In addition, the first chemical and spectroscopic characterization of the polymeric polyphenols, which account for over 50% of the resin, was also performed.

3.2 Extraction and Isolation

Plant Material The resin of *Dracaena cochinchinensis* was purchased at local market in Yunnan Province, China, in 2012, and identified by Prof. Hai-Zhou Li at Kunming University of Science and Technology. A voucher specimen (RDC201301) was deposited in the Laboratory of Natural Product Chemistry, Graduate School of Biomedical Sciences, Nagasaki University, Japan.

Extraction and Separation The resin (450 g) of *D. cochinchinensis* was dissolved in MeOH and insoluble precipitates were removed by filtration. The MeOH soluble part (432 g) was partitioned between hexane and MeOH. The MeOH layer (414.89 g) was separated by Sephadex LH-20 CC (10 cm i.d. × 40 cm, 100% ~ 60% EtOH, 50% acetone) to give five fractions. Fr. 2 was suspended in CHCl₃ to give CHCl₃ soluble part (Fr. 2-1, 64.0 g) and insoluble precipitates (Fr. 2-2, 228.0 g). The CHCl₃ soluble part was separated by Silica gel CC (8 cm i.d. × 20 cm, CHCl₃-MeOH 100:0 ~ 90:10 ~ 0:100) into five subfractions. Fr. 2-1-2 was further subjected to successive CC using Chromatorex ODS, silica gel and preparative HPLC to give 7,4'-dihydroxy-8-methylflavane (**3**),¹⁸ 5,4'-dihydroxy-7-methoxy-6-methylflavane (**4**),¹⁹ cochinchinenene G (**12**), cochinchinenene A (**16**)²⁹ and 7,4'-homoisoflavane (**24**)²⁷. Separation of Fr. 2-1-4 by CC using MCI gel, Sephadex LH-20, silica gel, Chromatorex ODS and preparative HPLC to yield 4,4'-dihydroxy-2,6-dimethoxy dihydrochalcone (**10**),²¹ loueirin C (**19**),²⁷ 4,4'-dihydroxy-2'-methoxychalcone (**20**),³⁰ 4,4'-dihydroxy-3'-methoxychalcone (**21**),³¹ 10,11-dihydroxydracaenone C (**25**),²⁷ 7,4'-dihydroxyflavanone (**26**),³² 7,4'-dihydroxyhomoisoflavanone (**27**),²⁷ 3',7-dihydroxy-4'-methoxyflavone (**28**),³³ (-)-(7'S,8S,8'R)-7',9-epoxy-4,4',9'-trihydroxy-3,3',5,5'-tetramethoxylignan-7-one (**30**),³⁴ secoisolariciresinol (**31**),³⁵ dihydrodehydroconiferyl alcohol (**32**),³⁶ and 5-methoxydihydrodehydroconiferyl alcohol (**33**).³⁷ A portion (145.0 g) of the CHCl₃ insoluble part (Fr. 2-2) was subjected to Sephadex LH-20 column (10 cm i.d. × 40 cm, 60% ~ 100% MeOH, 60% ~ 80% acetone) to give five fractions. Fr. 2-2-1 ~ 2-2-2

contained steroids and Fr. 2-2-3 ~ 2-2-5 contained phenolic substance. Fr. 2-2-1 was subjected to MCI gel and silica gel column to yield spirosta-5,25(27)-diene-1 β ,3 β -diol (neuroscogenin) 1-*O*-[*O*- α -L-rhamnopyranosyl-(1,2)- α -L-arabinopyranoside] (**34**)³⁸ and (25*R*)-spirost-5-en-3-ol-3-*O*- α -L-rhamnopyranosyl-(1,2)-[β -D-glucopyranosyl-(1,3)]- β -D-glucopyranoside (**35**).²⁸ Fr. 2-2-3 was applied to Diaion HP 20SS, silica gel, Sephadex LH-20 and Chromatorex ODS to yield **13**, cochinchinenene D (**14**),²⁹ cochinchinenene C (**15**),²⁹ 1-[5-(4,4'-dihydroxy-2-methoxydihydrochalconyl)]-1-(4-hydroxyphenyl)-3-(4-hydroxy-2-methoxyphenyl)propane (**17**),³⁹ cochinchinenin (**18**),⁴⁰ resveratrol (**22**),⁴¹ 3-*O*-methyl resveratrol (**23**),⁴² and 7,4'-dihydroxyflavone (**29**).⁴³ TLC and HPLC analysis showed Fr. 2-2-4, Fr.2-2-5 and Fr. 3 ~ Fr. 5 contained polymeric polyphenols. Fr. 2-2-4 (10.18 g) was dissolved in a solution consisting of 2-mercaptoethanol (50 mL), 0.3% HCl (400 mL) and EtOH (600 mL), and the resulting mixture was heated at 80 °C for 14 h. The mixture was purified over a Sephadex LH-20 column eluting with EtOH to give fractions containing a mixture of degradation products (Fr. 2-2-4-2, 2.46 g) and polymeric materials (Fr. 2-2-4-3, 4.79 g). Fr. 2-2-4-2 was further purified by CC over silica gel eluting with CHCl₃-MeOH-H₂O (90:10:1, 85:15:1 and 80:20:2, v/v/v) to give 7 fractions. Fr. 2-2-4-2-3 was subjected to Chromatorex ODS CC (50 ~ 100% MeOH) to give **37**, **38**, and pinosylvin (**40**).⁴⁴ Separation of Fr. 2-2-4-2-4 with Chromatorex ODS afforded **36**. Similar separation of Fr. 2-2-4-2-6 gave **39** (Chart 5).



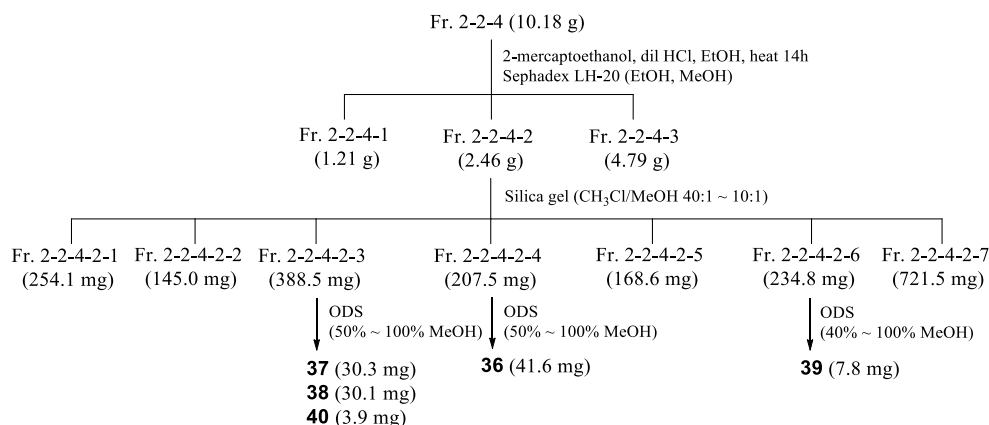
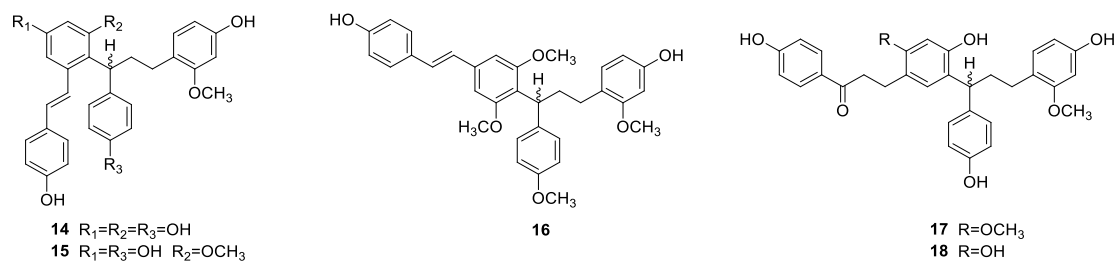


Chart 5. Extraction and separation from the red resin of *D. cochinchinensis*

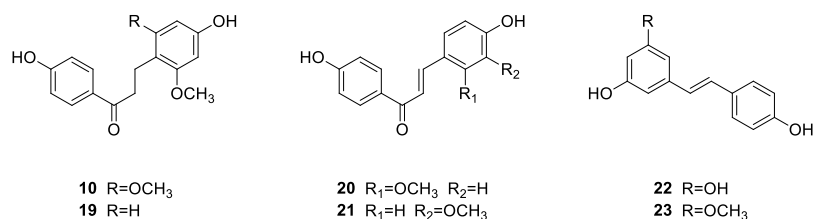
3.3 Structures of known compounds

In this study, 2 new and 25 known compounds were isolated and the known compounds were identified by spectroscopic comparison to the data in the literatures. The structures of the known compounds are shown in the following Figure 11.

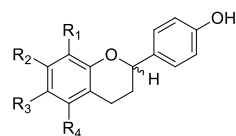
Flavonoid derivatives



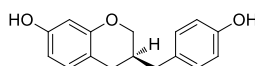
Chalcones and Stilbenes



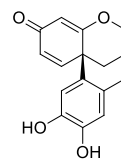
Flavanes and Homoisoflavanes



- 3 R₁=CH₃ R₂=OH R₃=R₄=H
 4 R₁=H R₂=OCH₃ R₃=CH₃ R₄=OH

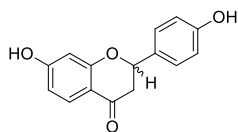


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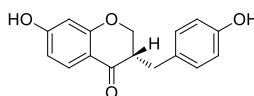


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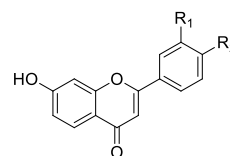
Flavones



26

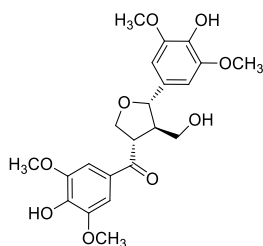


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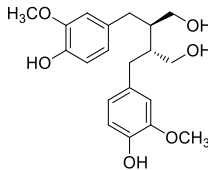


- 28 R₁=OH R₂=OCH₃
 29 R₁=H R₂=OH

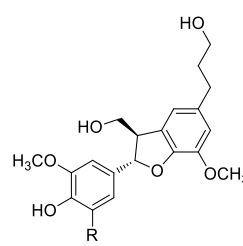
Lignans



30

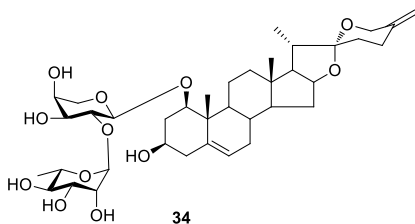


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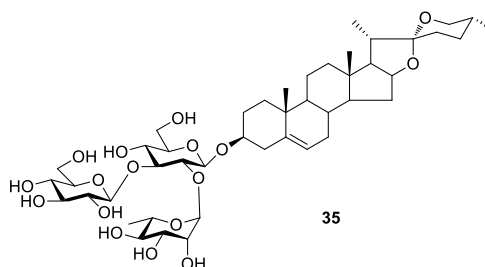


- 32 R=H
 33 R=OCH₃

Steroid saponins



34



35

Figure 11. Structures of known compounds isolated from Chinese dragon's blood.

3.4 Structure elucidation of new compounds

1) Cochinchinenene G (**12**)

Compound **12** was obtained as a yellow amorphous powder, and the molecular formula was determined to be $C_{33}H_{34}O_6$ by HR-FAB-MS, which showed the $[M+H]^+$ peak at m/z : 527.2437 (calcd for $C_{33}H_{35}O_6$: 527.2434). The 1H -NMR (Figure 12) and 1H - 1H COSY spectra of **12** showed two pairs of two-proton doublets [δ 7.13 (2H, d, $J = 8.2$ Hz), 6.73 (2H, d, $J = 8.2$ Hz), 7.13 (2H, d, $J = 8.2$ Hz), 6.73 (2H, d, $J = 8.2$ Hz)] arising from two 1,4-disubstituted aromatic rings. In addition, a set of ABX coupled signals at δ 6.71 (1H, d, $J = 8.2$ Hz), 6.33 (1H, d, $J = 2.3$ Hz), and 6.23 (1H, dd, $J = 8.2, 2.3$ Hz) was ascribable to a 1,2,4-trisubstituted aromatic ring, and *meta*-coupled signals at δ 6.64 (1H, d, $J = 1.8$ Hz) and 6.42 (1H, d, $J = 1.8$ Hz) indicated occurrence of a 1,2,3,5-tetrasubstituted aromatic ring (Figure 12). Besides the aromatic protons, signals attributable to an isolated *trans*-alkene group was appeared at δ 6.94 (1H, d, $J = 16.0$ Hz) and 6.70 (1H, d, $J = 16.0$ Hz)]. Moreover, aliphatic proton signals of two methylene groups [δ 2.29 (2H, m), 2.47 (2H, m)], one methine group [δ 4.54 (1H, br s)], and four methoxy groups [δ 3.77, 3.68, 3.67, 3.61, each (3H, s)] were observed. The ^{13}C -NMR and HSQC spectra exhibited signals attributable to 11 aromatic quaternary carbons including six oxygen bearing ones, 13 aromatic methine carbons, 3 aliphatic methine carbons, two aliphatic methylene carbons, and four methoxy carbons (Table 2).

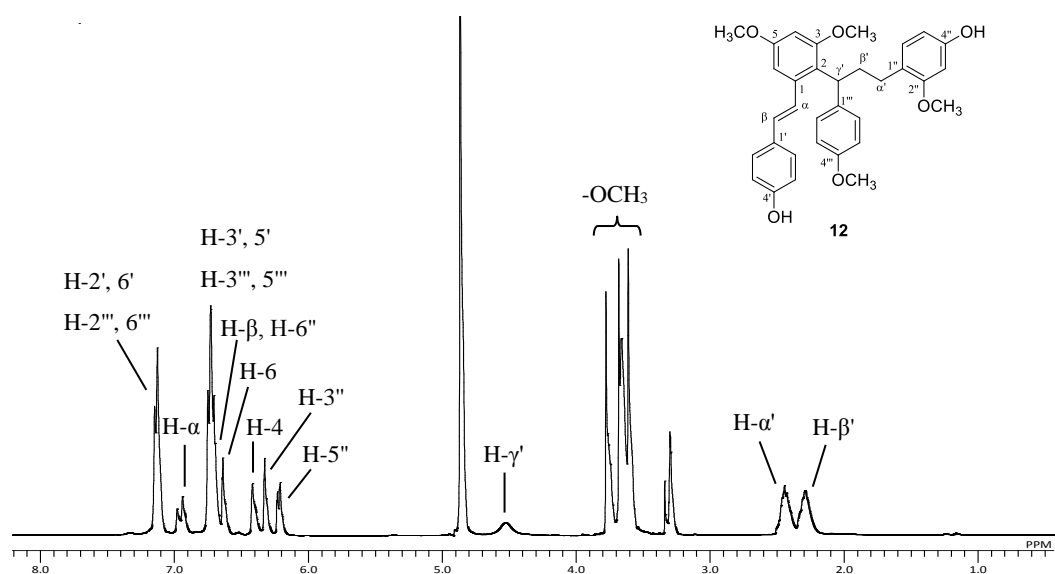


Figure 12. $^1\text{H-NMR}$ spectrum of **12** (400 MHz, in CD_3OD).

In the HMBC spectrum (Figure 13), correlations of the *trans*-alkene sp^2 protons to *p*-hydroxybenzene and tetra-substituted benzene rings revealed presence of a stilbene unit. Presence of an aliphatic chain composed of the C- α' , C- β' , and C- γ' was apparent from $^1\text{H-}^1\text{H}$ COSY correlations and connectivity of 4-hydroxy-2-methoxybenzene to the C- α' was revealed by observation of HMBC correlations of the H- α' . Although the H- γ' signal at δ 4.54 showed no HMBC correlation due to its severe broadening, NOESY correlations of the H- γ' to *trans*-alkene unit and *p*-methoxybenzene ring indicated the aforementioned stilbene unit was connected to the C- γ' of a chalcane unit. Location of the methoxy groups at the C-3, 5, 2'' and 4''' was determined by HMBC correlations, and it was confirmed by NOESY experiment. Based on these spectroscopic evidences, the structure of **12** was determined and named as cochinchinenene G. The compound name succeeded to those of the previous related compounds.²⁹ The severe broadening of the signals of H- γ' and C- γ' was observed and this was accounted for by restricted rotation of the linkage between C-2 and C- γ' carbons due to the presence of large substituents at neighboring positions. The very

small specific rotation value ($[\alpha]_D^{21} -1.6^\circ$) suggested that this compound was a racemic mixture.

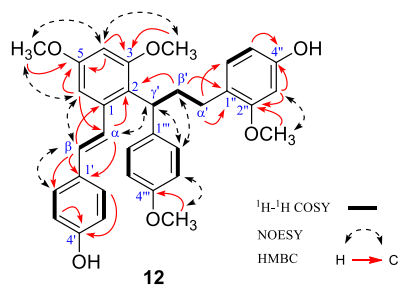


Figure 13. Selected ^1H - ^1H COSY, NOESY, and HMBC correlations of **12**.

2) Cochinchinenene H (**13**)

The molecular formula of compound **13** was confirmed to be $\text{C}_{30}\text{H}_{28}\text{O}_6$ by HR-FAB-MS, which exhibited the $[\text{M}+\text{H}]^+$ peak at m/z : 485.1961 (calcd for $\text{C}_{30}\text{H}_{29}\text{O}_6$: 485.1964). The ^1H -NMR spectrum (Figure 14) was related to that of **12**, showing two sets of A_2B_2 -type aromatic signals of *p*-oxygenated benzene rings, a set of ABX-type aromatic signals due to a 1,2,4-trisubstituted benzene, and a two-proton singlet signal attributable to a symmetrical 1,3,4,5-tetra-substituted benzene ring (Figure 14). Appearance of signals due to *trans*-alkene protons [δ 6.75 and 6.91 (each 1H, d, $J = 16.2$ Hz)] and a set of signals attributable to an aliphatic methine [δ 4.57 (1H, t, $J = 8.2$ Hz, H- γ')] and two methylene protons [δ 2.36 (2H, m, H- α') and 2.27 (2H, m, H- β')] are also similar to those of **12** (Table 2), suggesting that this compound is also a chalcane-stilbene conjugate (Figure 15). An apparent differences were presence of only one methoxy signal [δ 3.73 (3H, s)] and symmetric structure of the tetra-substituted benzene ring. The ^{13}C -NMR signals also supported these observations.

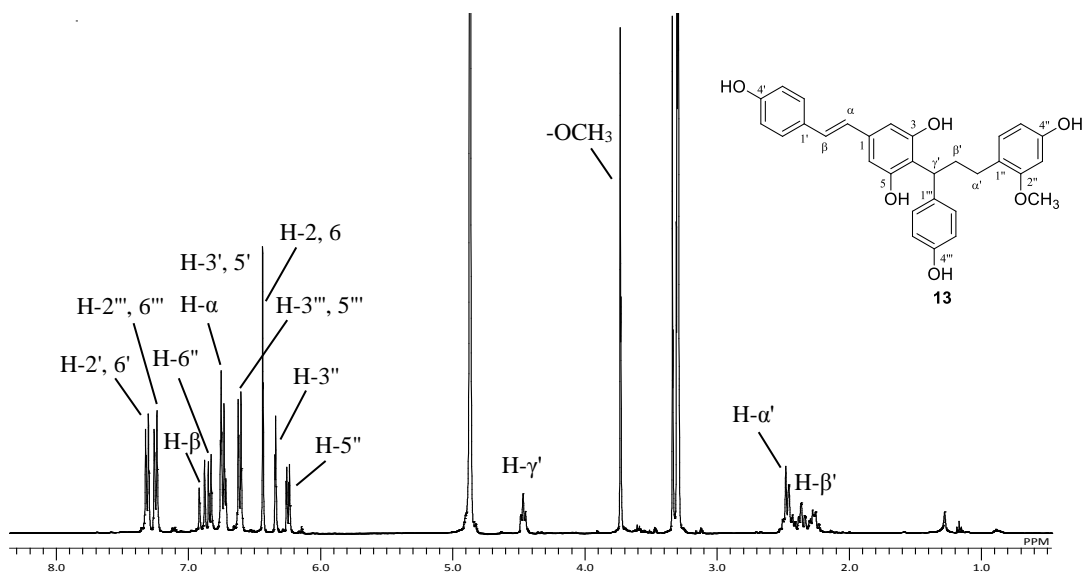


Figure 14. $^1\text{H-NMR}$ spectrum of **13** (400 MHz, in CD_3OD).

In the HMBC spectrum, aromatic carbons of the symmetrical tetra-substituted benzene ring showed cross peaks with *trans*-alkene protons, which also correlated to carbons of a *p*-hydroxybenzene ring, confirming presence of a stilbene unit (Figure 15). Absence of correlation of methoxy group with these two aromatic rings indicated that the stilbene unit is resveratrol. The HMBC correlation of the C-3, C-4, and C-5 of the resveratrol unit with the aliphatic methine proton (δ 4.47, H- γ') indicated chalcane unit was attached to the C-4 of the resveratrol moiety. Position of the methoxy group at the C-2'' of the trisubstituted benzene ring was determined by its HMBC correlation. Furthermore, the connection of the C- α' methylene to the C-1'' of the tri-substituted benzene and linkage of the C- γ' to another *p*-hydroxybenzene ring was also confirmed by HMBC and NOESY experiments. This compound is also supposed to be a racemic mixture due to its small specific rotation value ($[\alpha]_{\text{D}}^{21} -0.7^\circ$). Thus, the structure of **13** was concluded and named as cochinchinenene H.

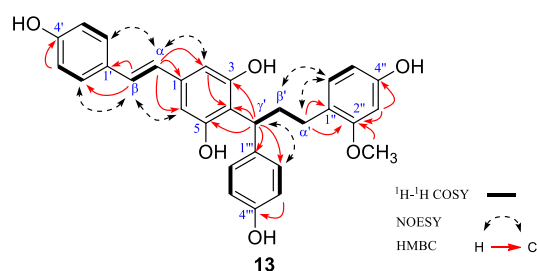


Figure 15. Selected ¹H-¹H COSY, NOESY and HMBC correlations of **13**.

Table 2. ¹H-(500MHz) and ¹³C-(125MHz) NMR data for compounds **12-13** (in CD₃OD)^a

position	12		13	
	δ_H	δ_C	δ_H	δ_C
1		140.4		130.6
2		125.2	6.44 (1H, s)	106.1
3		160.5		157.9
4	6.42 (1H, d, $J = 1.8$ Hz)	99.0		119.1
5		160.1		157.9
6	6.64 (1H, d, $J = 1.8$ Hz)	104.2	6.44 (1H, s)	106.1
α	6.94 (1H, d, $J = 16.0$ Hz)	126.4	6.75 (1H, d, $J = 16.2$ Hz)	127.0
β	6.70 (1H, d, $J = 16.0$ Hz)	131.2	6.91 (1H, d, $J = 16.2$ Hz)	128.3
1'		130.6		137.8
2', 6'	7.13 (2H, d, $J = 8.2$ Hz)	128.7	7.32 (2H, d, $J = 8.5$ Hz)	128.6
3', 5'	6.73 (2H, d, $J = 8.2$ Hz)	116.4	6.74 (2H, d, $J = 8.5$ Hz)	116.4
4'		158.0		158.1
1''		122.8		123.8
2''		159.7		159.6
3''	6.33 (1H, d, $J = 2.3$ Hz)	99.7	6.34 (1H, d, $J = 2.3$ Hz)	99.6
4''		157.5		157.3
5''	6.23 (1H, dd, $J = 8.2, 2.3$ Hz)	107.4	6.26 (1H, dd, $J = 8.2, 2.3$ Hz)	107.4
6''	6.71 (1H, d, $J = 8.2$ Hz)	131.1	6.84 (1H, d, $J = 8.2$ Hz)	131.1
α'	2.47 (2H, m)	29.1	2.36 (2H, m)	29.7
β'	2.29 (2H, m)	34.0	2.27 (2H, m)	34.5
γ'	4.54 (1H, br s)	40.1	4.47 (1H, t, 8.2)	41.0
1'''		138.5		138.3
2''', 6'''	7.13 (2H, d, $J = 8.2$ Hz)	129.6	7.25 (2H, d, $J = 8.2$ Hz)	130.2
3''', 5'''	6.73 (2H, d, $J = 8.2$ Hz)	114.1	6.62 (2H, d, $J = 8.2$ Hz)	115.2
4'''		158.6		155.6
3-OCH ₃	3.67 (3H, s)	55.6		
5-OCH ₃	3.77 (3H, s)	55.7		
2''-OCH ₃	3.61 (3H, s)	55.5	3.73 (3H, s)	55.6
4'''-OCH ₃	3.68 (3H, s)	55.6		

^a Chemical shifts are given δ values, multiplicities and coupling constants (J in Hz) in parentheses.

3.5 HPLC analysis of Chinese Dragon's blood

HPLC analysis of Chinese Dragon's Blood showed that the major constituents of this resin were detected as a large broad hump on base line (Figures 16 and 17). The chromatographic properties were similar to the black tea thearubigins⁴⁵⁻⁴⁶ and polymeric proanthocyanidins,⁴⁷ which are both polymers of coexisting monomeric flavan-3-ols.

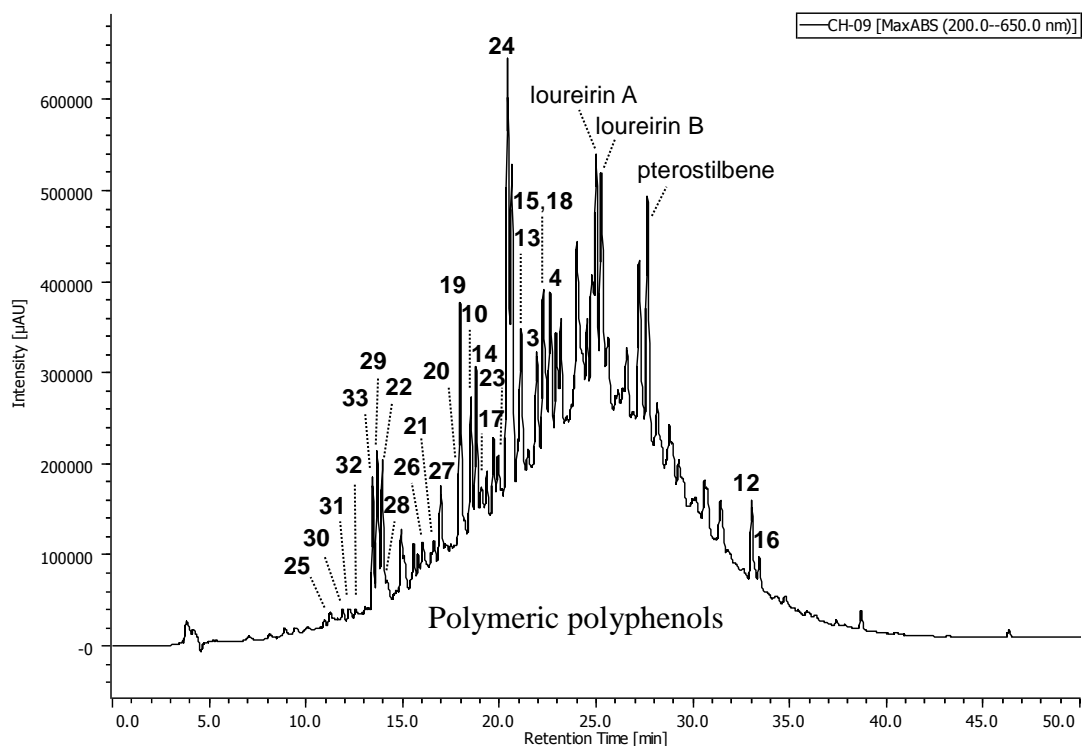


Figure 16. HPLC profile of Chinese Dragon's blood produced from *Dracaena cochinchinensis* (detection at max UV absorbance. Loureirin A, B and pterostilbene were identified by co-HPLC and comparison of UV absorption with authentic samples previously isolated from the same plant source.)

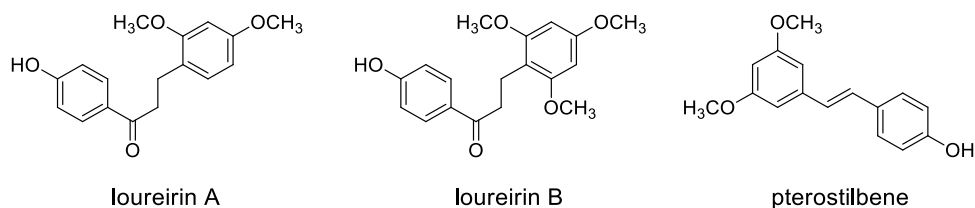


Figure 17. Structures of loureirin A, B and pterostilbene

3.6 Characterization of polymeric polyphenols of Chinese Dragon's blood

3.6.1 ^{13}C -NMR spectrum of polymer fraction

The fraction 5, which was strongly adsorbed on Sephadex LH-20 gel and eluted out with aqueous acetone contained only polymeric substance detected as a broad hump on HPLC base line (Figure 18). The ^{13}C -NMR spectrum of the fraction showed broad peaks closely related to those of chalcane unit of **17**, a chalcone-chalcane dimer isolated in this study. This observation strongly suggested that the polymer was mainly constituted of chalcane units. Very small signals attributable to a conjugated carbonyl carbon (δ 202) and an oxygenated aromatic carbon (δ 163) (arrows in Figure 19) that were similar to 4-hydroxyphenyl-keto moiety of the chalcone unit of **17** were detected when the spectrum was largely expanded, indicating that chalcone units with carbonyl group are contained in the polymer, although the contribution to polymerization is very low. Signals characteristic to stilbene units as those of **12** and **13** were not observed in the spectrum.

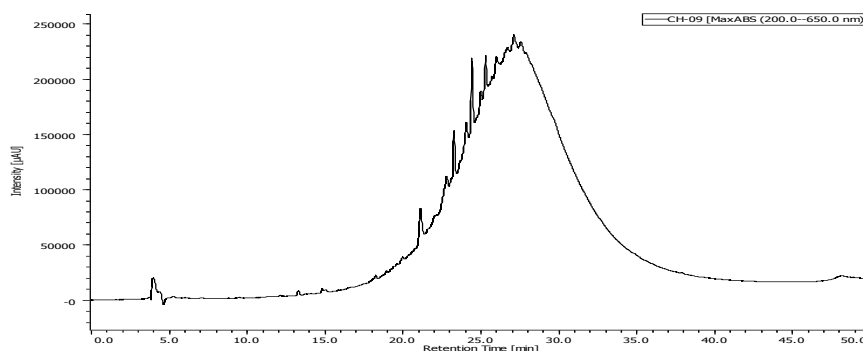


Figure 18. HPLC profile of Fr. 5.

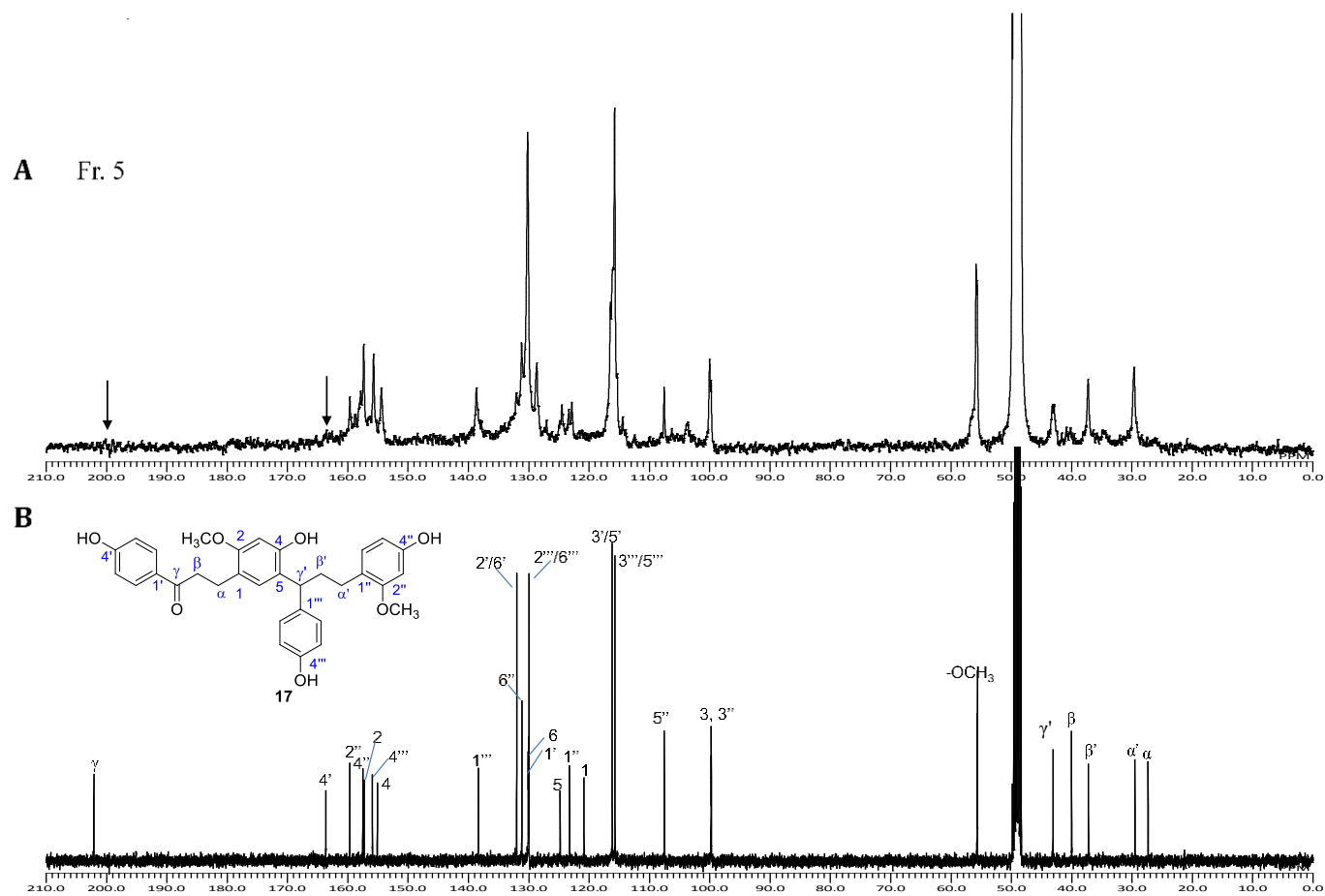


Figure 19. ^{13}C -NMR spectra of fraction 5 containing polymeric polyphenols and compound 17 (A: ^{13}C -NMR spectrum of Fr. 5. B: ^{13}C -NMR spectrum of 17).

3.6.2 Thiol degradation of polymer fraction

Thiol degradation is a method used for characterization of proanthocyanidins, in which substitution reaction with nucleophilic thiol compounds at methine carbon between two phenolic benzene rings cleaves inter-unit linkages (Figure 20).

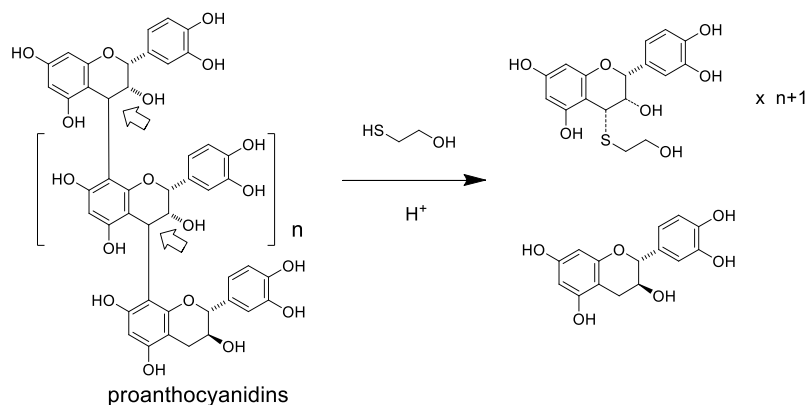
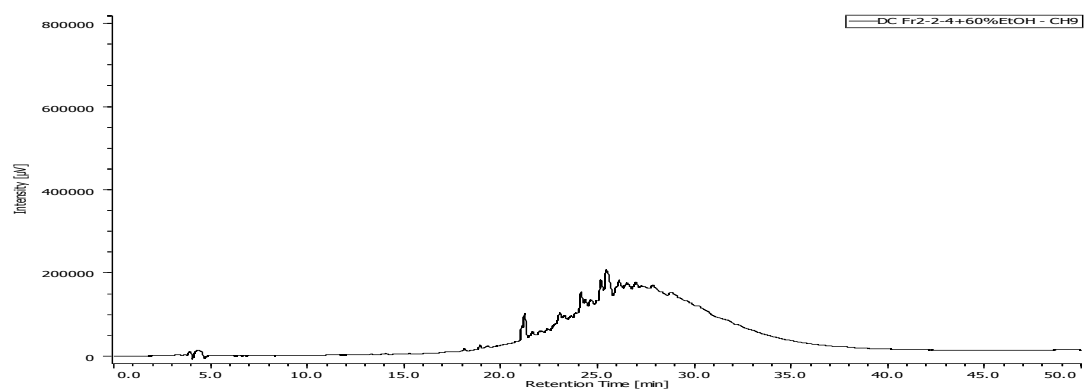


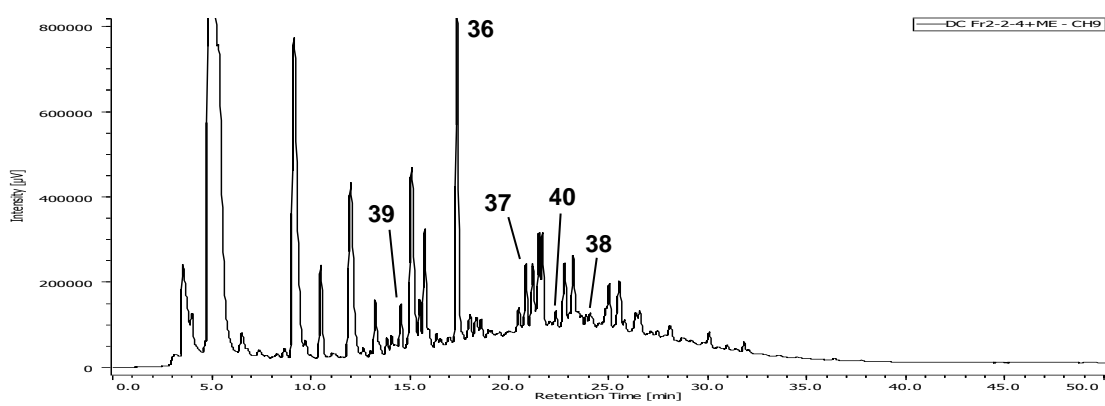
Figure 20. Thiol degradation of proanthocyanidins.

Thiol degradation of the polymeric fraction obtained from Chinese dragon's blood with mercaptoethanol under acidic conditions afforded **36**, confirming presence of 4-[3-(4-hydroxyphenyl)propyl]-3-methoxyphenol units in the polymers (Figure 21). The yield of **36** was very low compared to that expected from the ^{13}C -NMR spectrum. This was accounted for that the degradation method is not effective to the polymer of dragon's blood. Detail investigation of minor products yielded compounds shown in the Figure 22. Products **37** and **38** were byproducts of **36**, and isolation of **39** and **40** suggested presence of stilbene units in the polymer.

Before degradation



After thiol degradation (80 °C, 14 h)



Blank

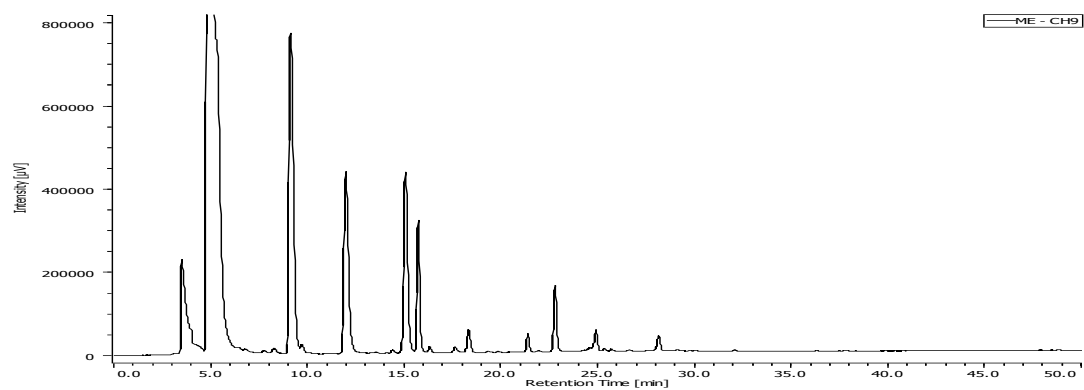


Figure 21. HPLC profile of the reaction mixture of thiol degradation of Fr. 2-2-4.

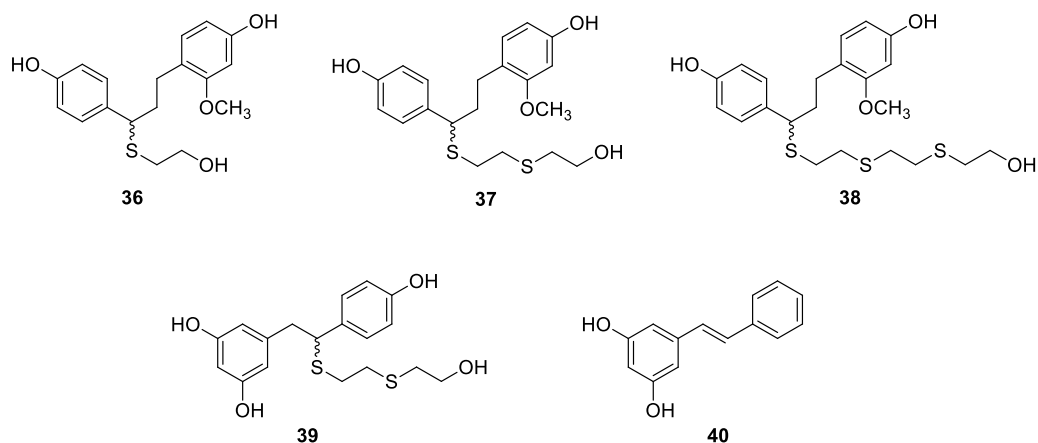


Figure 22. Minor thiol degradation products.

3.6.3 Methylation of polymer fraction

Based on the structures of chalcane dimers isolated in this study and the ^{13}C NMR spectrum of the polymer fraction, it was deduced that many of the phenolic hydroxy groups of the polymers are methylated. To reduce varieties of the structural analogues, the fractions containing oligomers and polymers were methylated with diazomethane. The ESI-MS of the methylated fractions indicated that at least heptamer of the flavonoid units were contained in the fractions (Figure 23); however, probably only a part of the components with relatively small molecular weights were detected in this ESI-MS experiment. Based on these observations, a plausible partial structure of polymeric flavonoid of Chinese dragon's blood was proposed as shown in Figure 24. This was the first chemical and spectroscopic evidence of the structural composition of polymeric flavonoids of Chinese dragon's blood.

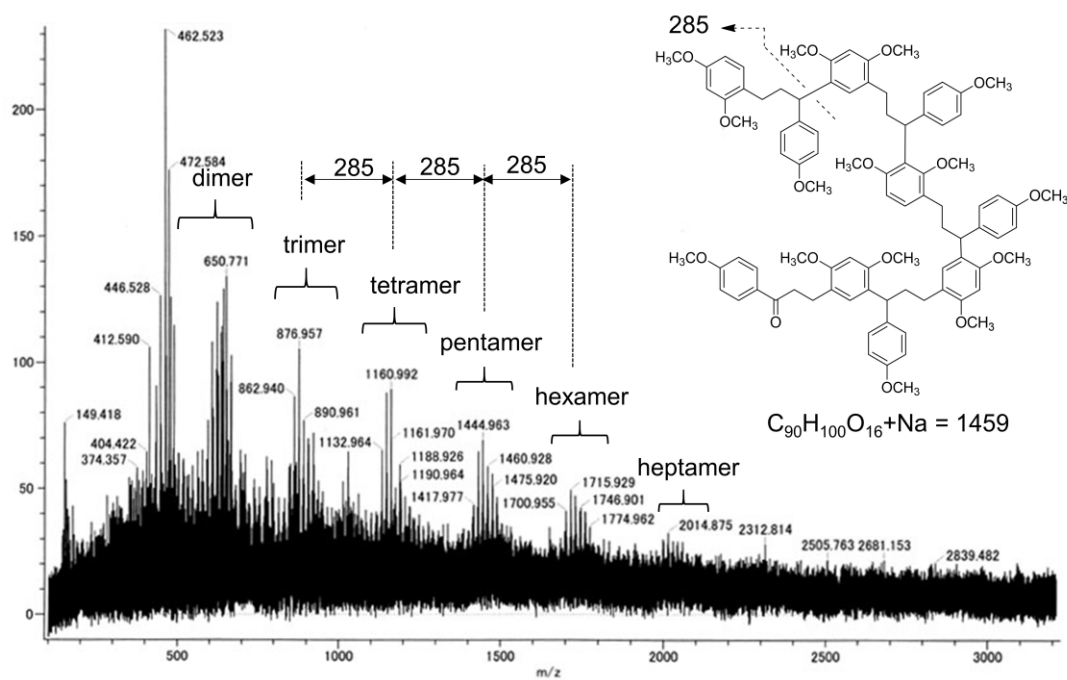


Figure 23. ESI-MS of the fraction 5 composed of polymeric polyphenols and a possible structure of pentamer (after methylation).

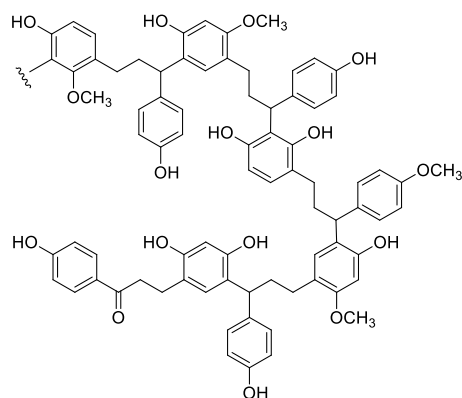


Figure 24. Structures of a plausible partial structure of oligomer.

3.7 Conclusion

Before our investigation, presence of 28 flavonoid and stilbene related compounds have been identified in Chinese dragon's blood.^{13,26,48-52} In the present study, we

isolated two additional new chalcane-stilbene conjugates (**12** and **13**) together with 25 known compounds. Furthermore, polymeric polyphenols, which have not been studied so far despite being the main constituents of the resin, were characterized by spectroscopic and chemical methods. The ^{13}C -NMR experiment, thiol degradation and methylation experiment indicated that the polymer was mainly composed of 4-[(4-hydroxyphenyl)propyl]-3-methoxyphenol units. This is the first chemical study on the polymeric substance and important from the viewpoints of not only traditional medicine but also plant defense system related to phytoalexin. Further study on chemical mechanism of oxidative oligomerization of chalcane are now in progress.

4 Reaction of essential oil and tea catechins in Cinnamon tea

4.1 Introduction

Flavored tea, which originated in China, produced by simple blending of tea products with fruits, flowers and spices. Typical examples are jasmine tea with flower of *Jasminum sambac*, Earl Grey with bergamot orange, and cinnamon tea with cinnamon. It has been cherished by Chinese and European people, and recently it also become popular in Japan.

Cinnamon tea produced by blending black tea with cinnamon bark. Cinnamon bark is an important spice used worldwide as well as one of the most important crude drugs in Oriental medicine and European herb. It contains essential oils and the major constituent is cinnamaldehyde (about 90%). Previous studies indicated cinnamaldehyde react with proanthocyanidins, which are catechin oligomers, under ambient conditions to give polymeric products.⁵³ Therefore, the chemical investigation of polymeric polyphenols of Cinnamon tea and reaction of cinnamaldehyde with epigallocatechin-3-*O*-gallate, the most abundant tea catechin, was examined.

4.2 HPLC analysis of Black tea and Cinnamon tea

HPLC analysis showed that chemical constituents of cinnamon tea and black tea are almost the same, except for appearance of a prominent peak of cinnamaldehyde (Figure 25). To confirm occurrence of reaction of tea catechin and cinnamaldehyde, commercial green tea was directly treated with cinnamaldehyde. The HPLC of the extract indicated decrease of tea catechins and production of polymeric substance detected as a broad hump on base line (Figure 26). The UV absorption of the broad hump was related to that of tea catechins. From this result, it was deduced that Cinnamon tea also contains polymeric polyphenols generated from tea catechins and cinnamaldehyde.

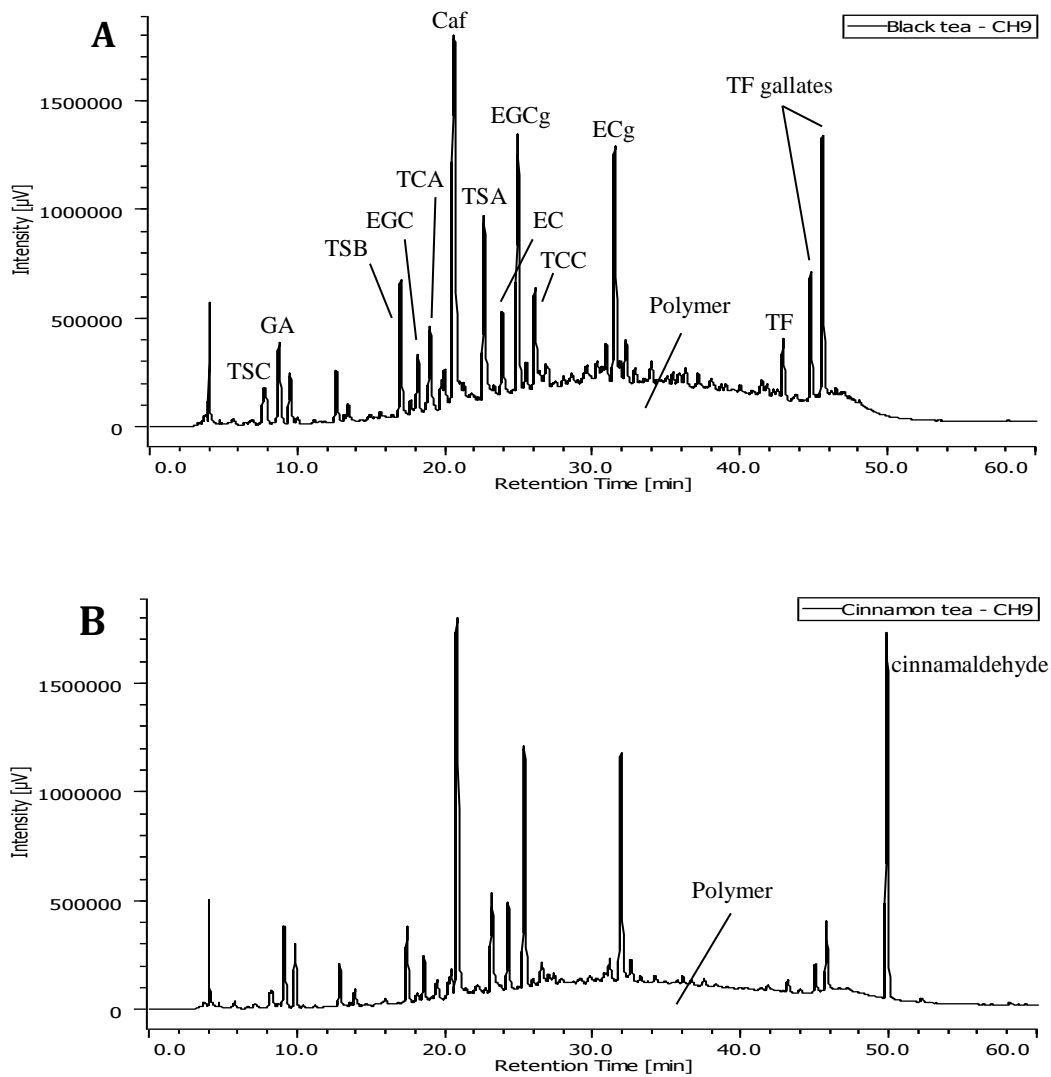


Figure 25. HPLC profiles of Black tea (A) and Cinnamon tea (B) extract (60% EtOH)

TSC: theasinensin C, GA: gallic acid, TSB: theasinensin B, EGC: epigallocatechin, TCA: theacitrin A, Caf: caffeine, TSA: theasnensin A, EC: epicatechin, EGCg: epigallocatechin-3-*O*-gallate, TCC: theacitrin C, ECg: epicatechin-3-*O*-gallate, TF: theaflavin, TF gallate: theaflavin-3-*O*-gallate, theaflavin-3'-*O*-gallate, theaflavin-3,3'-di-*O*-gallate, polymer: polymeric polyphenols including black tea thearubigins.

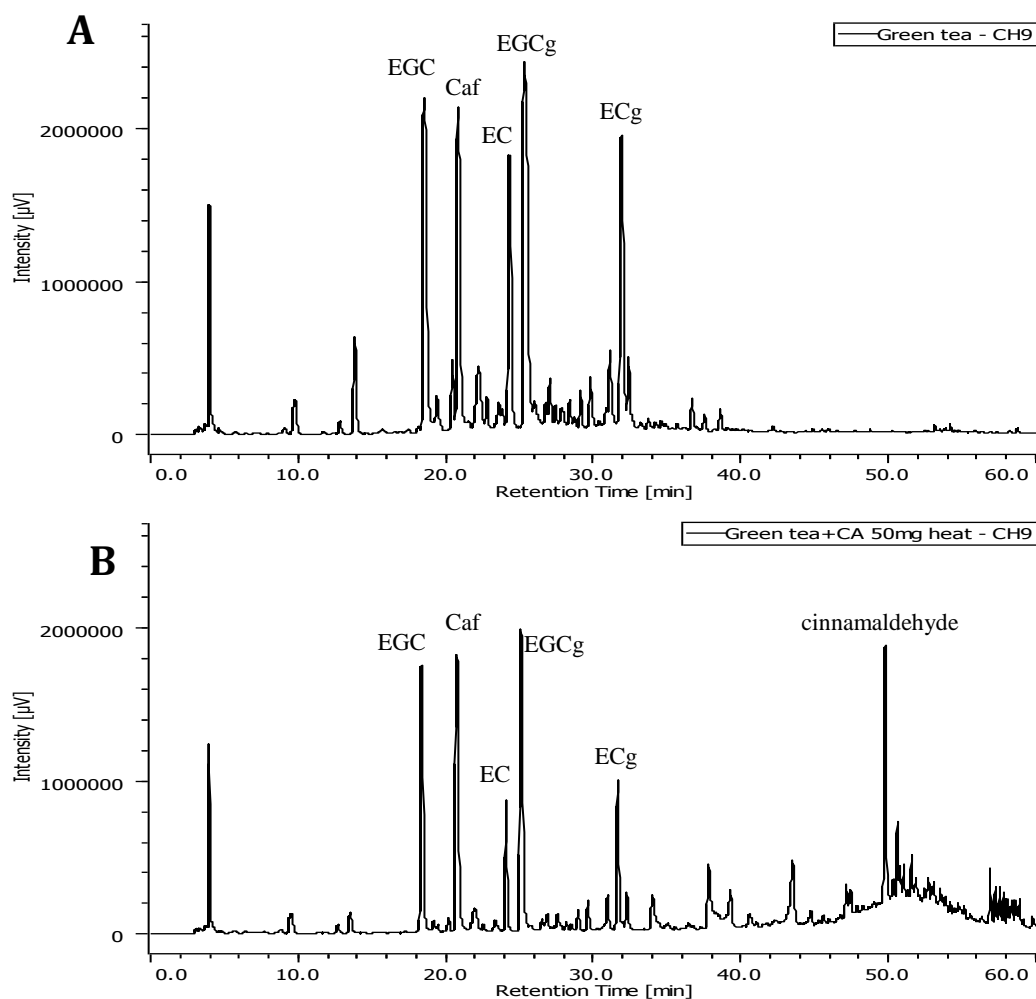


Figure 26. HPLC profiles of green tea treated with cinnamaldehyde.

(A) green tea leaves were extracted with 60% EtOH. (B) green tea (1 g) was mixed with 80% acetone solution (10 mL) containing cinnamaldehyde (50 mg) and evaporated under reduced pressure. Then, the tea leaves heated at 80 °C for 1 h.

4.3 Extraction and isolation of polymeric polyphenols from Cinnamon tea.

Cinnamon tea (50 g) was extracted with 60% acetone 3 times at room temperature. The extract was concentrated under reduced pressure until the organic solvent was removed and the resulting aqueous solution was partitioned with AcOEt. Size-exclusion chromatography of the AcOEt layer (1.0 g/5.9 g) was performed using Sephadex LH-20 column (2 cm i.d. \times 45 cm) with 7 M urea-acetone (4:6, v/v) to give

two fractions. Fraction A containing polymeric substance was applied to Diaion HP 20SS (2 cm i.d. × 18 cm, 30% ~ 100% MeOH) to yield polymeric polyphenols (22.4 mg). The precipitate (1.0 g/3.9 g) formed during solvent partitioning was separated by the same size-exclusion chromatography to give polymer fractions, C-1 – C-4 (Chart 6). The HPLC profile of the polymeric polyphenols was shown in Figure 27.

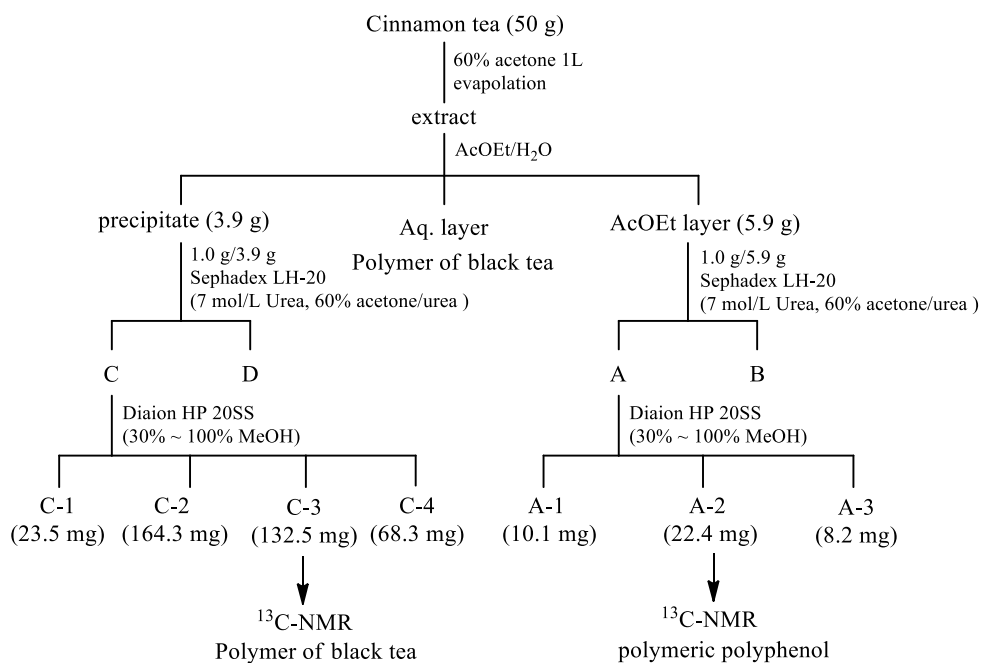


Chart 6 Separation of polymeric polyphenols from commercial Cinnamon tea

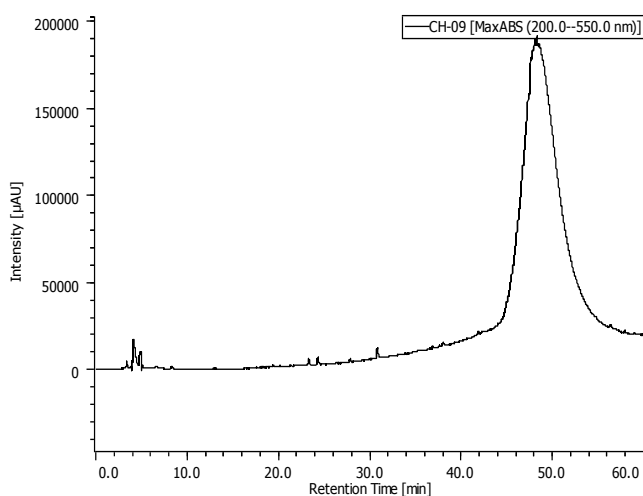


Figure 27. HPLC profile of polymeric polyphenol obtained from Cinnamon tea.

4.4 ¹³C-NMR spectrum of polymeric polyphenol

The backbone of the polymer of Cinnamon tea is black tea polymeric polyphenols because Cinnamon tea is produced by addition of cinnamon bark to black tea. The ¹³C-NMR spectrum of the polymeric polyphenol fraction obtained from cinnamon tea (Figure 28) showed signals ascribable to galloyl group, A-, B- and C-rings of flavan-3-ol units, which were related to polymeric polyphenols obtained from black tea.⁴⁶ The signals of B-ring 2,6 carbons at δ 106 ppm was relatively small compared to the ¹³C NMR spectrum of epigallocatechin-3-*O*-gallate. This is because oxidative coupling of B-rings is the major reaction mechanism of the polymerization of tea catechins during black tea production, which is enzymatic oxidation so-called tea-fermentation.⁵⁴ The spectroscopic difference between the polymers of Cinnamon tea and black tea was appearance of signals assignable to phenyl group of cinnamaldehyde at δ 128 ppm (Figure 28). In addition, signals at δ 90 ~ 100 ppm due to C-6, 8 of flavan-3-ol A-ring were smaller than those of epigallocatechin-3-*O*-gallate. The spectroscopic observation suggested that the polymeric polyphenols of Cinnamon tea was condensation products of black tea polymeric polyphenols and cinnamaldehyde.

Our previous research showed that cinnamaldehyde easily reacts with A-rings of flavan-3-ol units to give polymerization products. In fact, the polymeric procyanidins in the cinnamon bark are complex mixture of procyanidin-cinnamaldehyde condensation products with anthocyanidin structures.⁵³ In this study, we examined occurrence of the similar reaction of tea catechins and cinnamaldehyde in cinnamon tea.

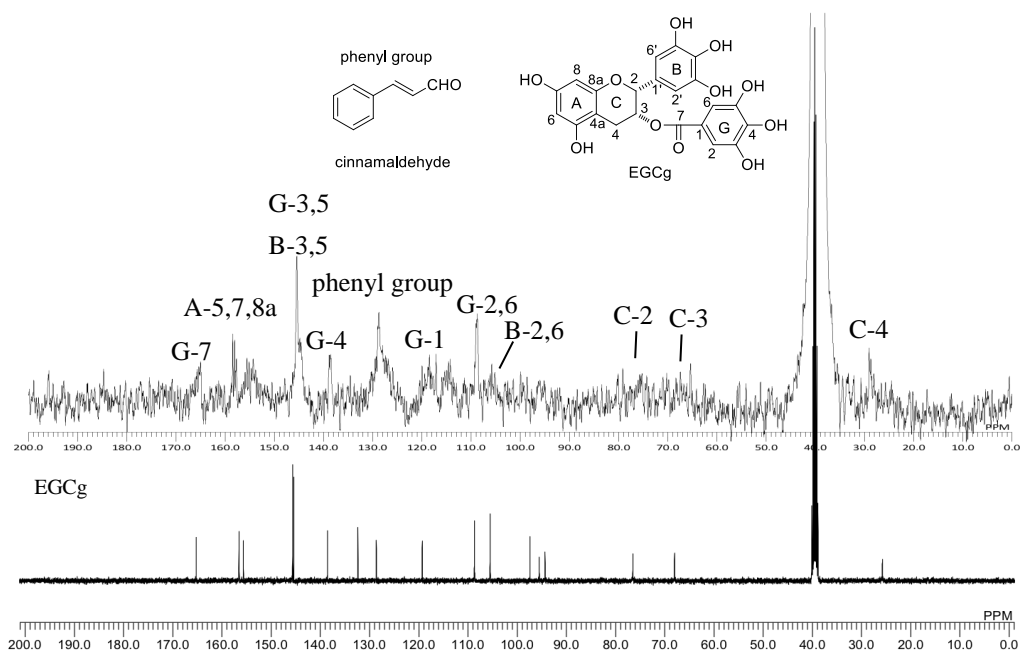


Figure 28. ^{13}C -NMR spectrum of polymeric polyphenols obtained from cinnamon tea (in $\text{DMSO-}d_6$).

4.5 Reaction of (-)-epigallocatechin-3-*O*-gallate (EGCg) and cinnamaldehyde

(-)-Epigallocatechin-3-*O*-gallate (**41**, 10 g) and cinnamaldehyde (**42**, 2.9 g) (Figure 29) were dissolved in EtOH (1% TFA, 40 mL) and kept at room temperature for 2 days. The mixture was separated into 7 fractions by Sephadex LH-20 column chromatography (4 cm i.d. \times 23 cm) with EtOH/ H_2O /acetone (0.1% TFA). Fraction 7 (1.0 g/11.8 g) was successively subjected to column chromatography using silica gel ($\text{CHCl}_3/\text{MeOH}/\text{H}_2\text{O}$, 90:10:1 \sim 60:40:10) and ODS (20% \sim 100% MeOH, 10% stepwise elution) to yield **43** (10.5 mg). The major products of this reaction were polymeric substance.

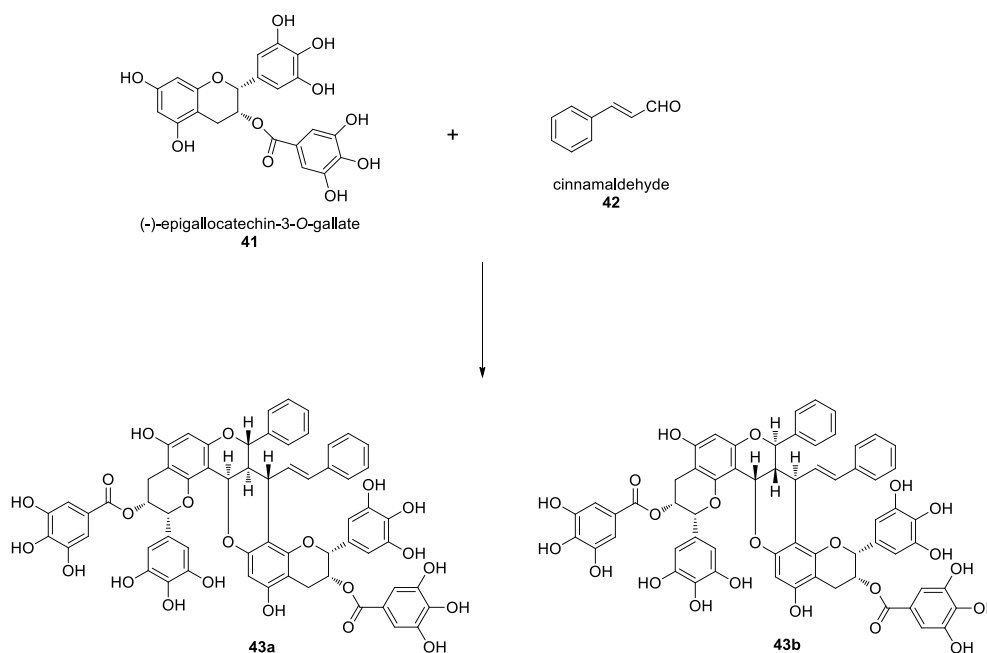


Figure 29. Structure of **41**—**43**.

The product **43** was obtained as a yellow amorphous powder and the molecular formula $C_{62}H_{48}O_{22}$ was confirmed by HR-FAB-MS (m/z 1167.2543, $[M+Na]^+$, Calcd for $C_{62}H_{48}O_{22}Na$: 1167.2536). The 1H - and ^{13}C -NMR spectrum (Table 3) showed that the presence of two skeletons of epigallocatechin-3-*O*-gallate (**41**) and two phenylpropanoid units originated from cinnamaldehyde (**42**). The 1H -NMR spectrum showed signals attributable to two monosubstituted benzene rings and a pair of *trans*-olefin protons (H-7' and H-8') (Figure 30). The two singlet signals due to the A ring of **41** indicated that **42** was attached to the C-6 or C-8 positions of **41**. Usually C-8 showed more effective than C-8. In this case, the possible reaction mechanism of **41** reacted with **42** to form **43** shown in Figure 31. The 1H - 1H COSY spectrum (Figure 30) showed the cross-peaks between methine protons H-9' and H-8''', and in turn H-8''' was coupled with two oxygen-bearing methane protons H-7''' and H-9'''. The connection of these four aliphatic methane carbons C-9', C-7''', C-8''' and C-9''' was confirmed by their HMBC correlations. The location of the two benzene rings at C-7' and C-7''' were apparent from their HMBC correlations (Figure 30). In the HMBC spectrum, H-9' correlated with flavan A-ring C-7, C-8 and C-8a, and H-9''' correlated

with C-7'', C-8'' and C-8a'' of another flavan-A-ring, indicating C-9' and C-9''' were attached to the C-8 and C-8'' positions, respectively.

Table 3. ¹H-(500 MHz) and ¹³C-(125 MHz) NMR data for compounds **43** (in acetone-*d*₆)^a

	δ_H	δ_C		δ_H	δ_C
2	5.20 (1H, brs)	78.0	2''	5.05 (1H, brs)	77.6
3	5.61 (1H, m)	69.1	3''	5.57 (1H, m)	68.6
4	3.15 (2H, m)	26.0	4''	3.14 (2H, m)	25.9
4a		100.1	4''a		99.8
5		155.9	5''		154.9
6	6.40 (1H, s)	96.9	6''	6.10 (1H, s)	95.8
7		153.2	7''		157.8
8		100.5	8''		103.3
8a		153.7	8''a		155.3
B-1		130.4	B-1''		130.5
B-2, 6	6.71 (2H, s)	106.8	B-2'', 6''	6.44 (2H, s)	106.7
B-3, 5		146.1	B-3'', 5''		145.9
B-4		133.0	B-4''		133.0
G-1		122.0	G-1''		121.8
G-2, 6	7.13 (2H, s)	110.2	G-2'', 6''	7.11 (2H, s)	110.0
G-3,5		145.8	G-3'', 5''		145.8
G-4		138.7	G-4''		138.7
G-7		166.1	G-7''		166.0
1'		138.4	1'''		140.3
2', 6'	7.20 (2H, d, <i>J</i> = 7.5 Hz)	127.0	2''', 6'''	7.51 (2H, d, <i>J</i> = 7.0 Hz)	128.1
3', 5'	7.14 (2H, brt, <i>J</i> = 7.5 Hz)	129.1	3''', 5'''	7.47 (2H, brt, <i>J</i> = 7.4 Hz)	129.5
4'	7.06 (1H, m)	127.6	4'''	7.37 (1H, m)	129.4
7'	6.14 (1H, d, <i>J</i> = 16.1 Hz)	133.8	7'''	5.07 (1H, d, <i>J</i> = 11.3 Hz)	77.6
8'	6.29 (1H, dd, <i>J</i> = 16.1, 5.9 Hz)	33.7	8'''	2.39 (1H, ddd, <i>J</i> = 11.3, 2.3, 1.4 Hz)	42.0
9'	3.53 (1H, d, <i>J</i> = 5.9 Hz)	77.6	9'''	5.58 (1H, d, <i>J</i> = 2.3 Hz)	62.6

^a Chemical shifts are given δ values, multiplicities and coupling constants (*J* in Hz) in parentheses.

Configuration of methane carbons were determined by NOESY correlations and ¹H-¹H coupling constants. The coupling constant between H-7''' and H-8''' (11.3 Hz) showed that the dihedral angle between these protons was almost 180°. In contrast, the coupling constant H-8''' with H-9' was 1.4 Hz. The NOESY correlations (Figure 30) showed NOEs between H-9' and H-7'''. Furthermore, the H-9''' showed NOEs with H-8' and H-8'''. These observations confirmed the relative stereochemistry aliphatic carbons originated from **42**; however, the absolute configuration could not be determined. Thus, the structure of this product was represented by **43a** or **43b** as

shown in Figure 29. The structure of this products suggested that tea catechins and black tea polymeric polyphenols are cross-linking of tea polyphenols including black tea polymeric polyphenols by condensation with cinnamaldehyde at the flavan-A-rings.

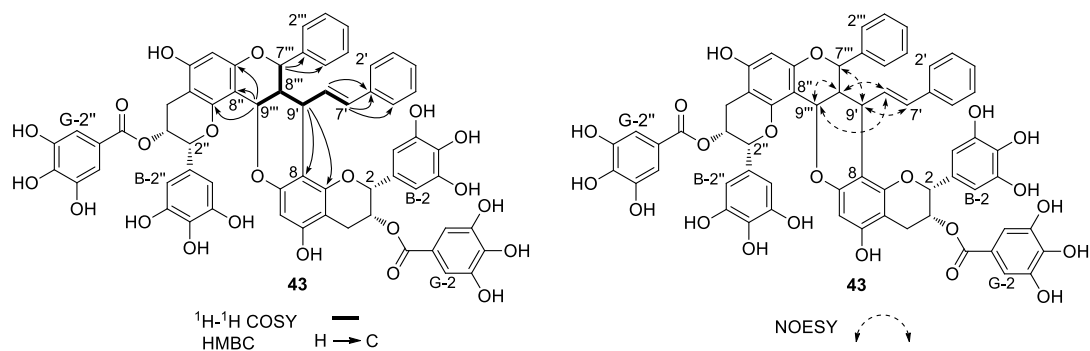


Figure 30. Selected ^1H - ^1H COSY, NOESY, and HMBC correlations of **43**.

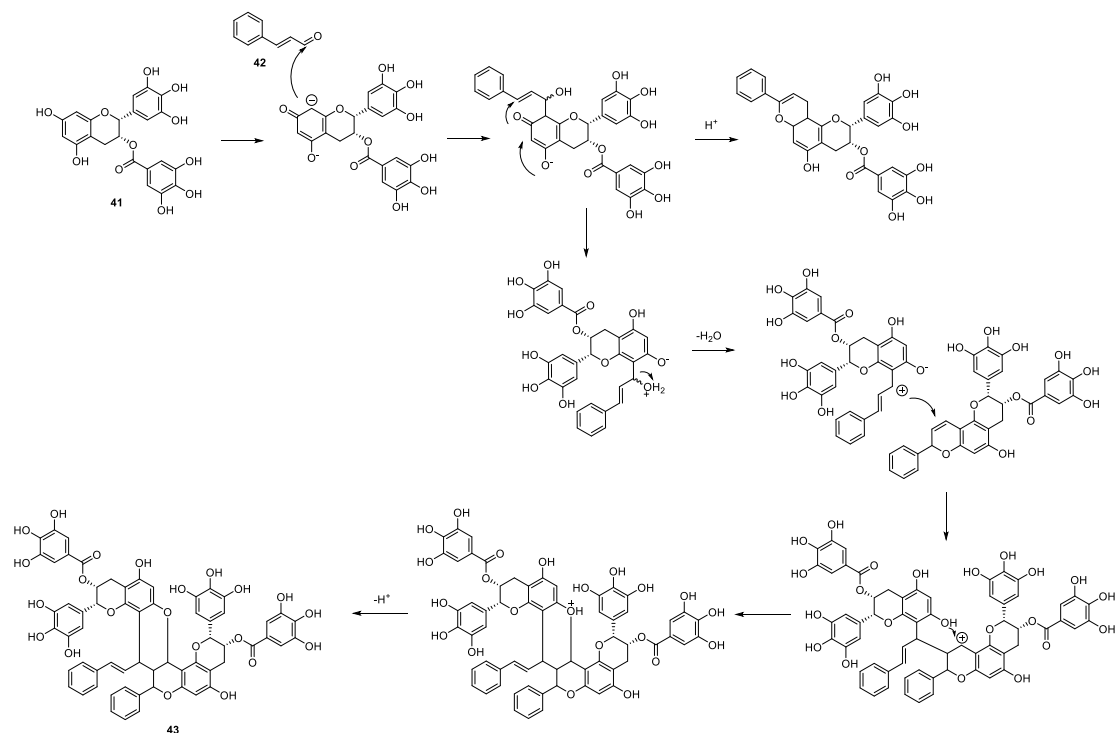


Figure 31. Possible reaction mechanism of **41** reacted with **42** to form **43**.

4.6 Conclusion

The study described in this chapter suggested that simple blending of cinnamon bark and black tea generate condensation products of tea polyphenols and cinnamaldehyde. Model experiments using green tea and **41** indicated that the reactions are complex cross-link polymerization and the major products are polymeric polyphenols. Purpose of addition of cinnamon bark to black tea is apparently addition of the characteristic flavor to usual black tea. However, the results obtained in this study suggest that decrease of tea catechins, which has bitter and astringent taste, should also be considered. In addition, recent biological studies indicated polymeric polyphenols shows strong inhibitions to digestive enzymes and decrease sugar and lipid uptake from digestive tract. Since reactions of tea catechins with other essential oils are also known, further chemicals studies on flavored black tea and green tea are necessary.

5 Summary and Conclusion

Dragon's blood is a red resin obtained from different species of four distinct plant genera, *Croton* sp. (Euphorbiaceae), *Pterocarpus* sp. (Leguminosae), *Daemonorops* sp. (Arecaceae) and *Dracaena* sp. (Agavaceae).³

Chinese dragon's blood is the red resin generated in the wounded wood of *Dracaena cochinchinensis*, which is a shrub distributed in the southwest of China. The resin has been used as the substitute for the imported dragon's blood produced from *Daemonorops draco* since 1970s.⁴⁻⁵ Despite traditional importance of these Dragon's blood, only a part of chemical constituents of these resins have so far been clarified. Therefore, in this study the commercial resins produced from *Daemonorops draco* and *Dracaena cochinchinensis* were investigated in detail.

Chapter 2 describes the chemical constituents of dragon's blood from *Daemonorops draco*. Three new flavans and eight known flavonoids were isolated in this study. The new compounds (**5-7**) are the first examples of 2-methoxyflavans. The major components were 7-hydroxy-5-methoxyflavan (**1**), 7-hydroxy-5-methoxy-6-methylflavan (**2**), and 4,6-dihydroxy-3-methyl-2-methoxychalcone (**11**). Interestingly, the location of the carbonyl group at the C-2 of **11** is different from that of usual chalcones produced by shikimate-polyketide pathway, which has the carbonyl group at the position adjacent to the phloroglucinol ring. Isolation of **5-7** suggested that the unusual dihydrochalcone **11** was produced from **2** via hydroxy analogue of **7**.

In Chapter 3, constituents of Chinese dragon's blood from wounded wood of *Dracaena cochinchinensis* were examined, and two new chalcane-stilbene conjugates (**12** and **13**) and 25 known compounds were isolated. The polymeric flavonoids have not been studied so far despite being the main constituents of the resin, accounting for over 50% of the total resin. In this study the polymeric substance was isolated and characterized by spectroscopic and chemical methods. The ¹³C-NMR experiment, thiol degradation, and ESI-TOF-MS of methylation products indicated that the

polymer was mainly composed of C-C-linked chalcone units, and the major component was found to be 4-[3-(4-hydroxyphenyl)propyl]-3-methoxyphenol. This is the first chemical study on the polymeric substance. The polymeric substance of Chinese dragon's blood was shown to be produced by bacterial and enzymatic reaction from the chalcones. The results are important from the viewpoints of not only traditional medicine but also plant defense system related to phytoalexin. Further study on the chemical and biological mechanism of the polymer production is needed.

There is no taxonomical relationship between *Daemonorops draco* and *Dracaena cochinchinensis* that are the source plants of two Dragon's blood samples examined in this study. However, chemical constituents of these resins are closely related and both resins contain flavans and chalcones as major polyphenols. This is very interesting from the viewpoint of ethno-pharmacology.

In Chapter 4, chemical significance of blending of herbs to tea products was verified. Flavored tea produced by simple blending of tea products with fruits, flowers and spices. In case of cinnamon tea, purpose of mixing cinnamon bark with black tea is apparently addition of the characteristic flavor to usual black tea. However, the results of this study suggested additional effects which may concern to decrease of astringency of tea catechins. The ^{13}C -NMR spectrum of polymeric polyphenol obtained from relatively non-polar fraction of cinnamon tea extract showed evidence of condensation of cinnamaldehyde with catechins. Furthermore, model reaction of cinnamaldehyde with epigallocatechin gallate at room temperature generate condensation product composed of two catechin units and two phenylpropanoid units. The results suggested that blending cinnamon bark to tea products may cause co-polymerization of tea catechin in the tea leaves and decrease the bitter and astringent taste of tea catechins.

In this thesis, two examples of polymeric polyphenols obtained from Chinese dragon's blood and cinnamon tea were examined; however, many of the related polymers in plant medicines used in traditional Chinese medicine, Japanese Kampo medicine, and European herbs have not been chemically characterized. Similar

polymeric polyphenols are also detected in many processed foods produced from fruits and vegetables. Recent biological studies suggested that the polymeric substance shows inhibition activities against digestive enzymes and decrease lipid and sugar uptake from digestive tract to blood flow.⁵⁵ Therefore, further chemical studies of the uncharacterized polymeric polyphenols are needed in future for understanding of relationship between the structure and the function.

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Experimental Section

General experimental procedures

IR and UV spectra were obtained with JASCO FT/IR-410 and JASCO V-560 UV/Vis spectrophotometers (JASCO Co., Tokyo, Japan), respectively; optical rotations were measured with a JASCO P-1020 digital polarimeter. ^1H and ^{13}C NMR spectra were measured in CD_3OD , CDCl_3 , $\text{DMSO}-d_6$ and $\text{acetone}-d_6$ at 27 °C using a Varian Unity plus 500 spectrometer (Varian, Palo Alto, CA) operating at 500 MHz for ^1H and 125 MHz for ^{13}C NMR experiments or a JEOL JNM-AL 400 spectrometer (JEOL Ltd., Tokyo, Japan) operating at 400 MHz for ^1H and 100 MHz for ^{13}C NMR experiments. Coupling constants are expressed in Hz and chemical shifts are given on a δ (ppm) scale with tetramethylsilane as an internal standard. ESI-MS were obtained using a JEOL JMS-T100TD spectrometer (JEOL Ltd., Tokyo, Japan). HR-FAB-MS were recorded on a JMS 700N spectrometer (JEOL Ltd., Tokyo, Japan), with *m*-nitrobenzyl alcohol or glycerol used as the matrix. Column chromatography (CC) was conducted with Silica gel 60N (100–210 μm , Kanto Chemical Co., Tokyo, Japan), Diaion HP20SS (Mitsubishi Chemical Co. Tokyo, Japan), MCI-gel CHP 20P (75–150 μm , Mitsubishi Chemical Co., Tokyo, Japan), Sephadex LH-20 (25–100 μm , GE Healthcare Bio-Science AB, Uppsala), and Chromatorex ODS (100–200 mesh; Fuji Silysia Chemical Ltd., Tokyo, Japan) columns. TLC was performed on precoated Kieselgel 60 F₂₅₄ plates (0.2 mm thick, Merck, Darmstadt, Germany) with toluene–ethyl formate–formic acid (1:7:1 and 1:5:2 v/v/v), CHCl_3 –MeOH– H_2O (7:3:0.5, 8:2:0.2 and 9:1:0.1 v/v/v) and hexane–AcOEt (5:1 v/v) mixtures being used as the eluents. Spots were detected by UV illumination (254 nm) and by spraying with 2% ethanolic FeCl_3 and 10% sulfuric acid, followed by heating. Preparative HPLC was performed on a Cosmosil 5C₁₈-PAQ column (20 mm i.d. \times 250 mm, Nacalai Tesque Inc., Kyoto, Japan) with 70% MeOH, 30%, 45% CH_3CN and a Cosmosil 5SL-II column (10 mm i.d. \times 250 mm, Nacalai Tesque Inc.) with

hexane-EtOAc (4:1). Analytical HPLC was performed on a 250 mm × 4.6 mm i.d. Cosmosil 5C₁₈-AR II column (Nacalai Tesque Inc.) with gradient elution of CH₃CN in 50 mM H₃PO₄ from 10% to 30% in 30 min and 30% to 75% in 15 min, 20% ~ 100% in 45min at a flow rate of 0.8 mL/min, and detection with a Jasco MD-910 photodiode array detector.

Chapter 2

Extraction and Isolation

D. draco resin (120 g, purchased in Guangdong, China, in 2012) was thoroughly suspended in CHCl₃ (1.0 L) and stirred at room temperature. The insoluble materials were removed by filtration and the filtrate was concentrated under reduced pressure to yield a CHCl₃-soluble fraction (104.8 g). The extract was subjected to CC on a silica gel column (8 cm i.d. × 25 cm, CHCl₃-MeOH-H₂O 100:0:0–90:10:1–80:20:2–0:100:0) to give four fractions: Fr. 1 (12.2 g), Fr. 2 (11.5 g), Fr. 3 (58.0 g), and Fr. 4 (5.8 g). Fractions 1 and 2 were shown to contain mainly terpenoids by TLC analysis. Fraction 3 was separated on a Sephadex LH-20 column (7 cm i.d. × 40 cm) with EtOH and then 60% acetone to give five fractions: Fr. 3-1 (22.19 g), Fr. 3-2 (13.46 g), Fr. 3-3 (18.23 g), Fr. 3-4 (1.56 g), and Fr. 3-5 (0.24 g). Fraction 3-3 was separated on a Diaion HP20SS column (5 cm i.d. × 20 cm) with MeOH-H₂O (40–100%, 20% stepwise elution) to give five fractions: Fr. 3-3-1 (1.91 g), Fr. 3-3-2 (3.42 g), Fr. 3-3-3 (3.33 g), Fr. 3-3-4 (6.37 g), and Fr. 3-3-5 (2.0 g). Fraction 3-3-1 was purified by column chromatography over silica gel (3 cm i.d. × 10 cm) with hexane-EtOAc (5:1–4:1–3:1–2:1–1:1) to give 10 subfractions. Subfraction 3-3-1-7 (741.1 mg) was purified using preparative reverse-phase HPLC and normal-phase HPLC to yield **3** (5.5 mg), **4** (6.6 mg), **7** (83.4 mg) **8** (6.6 mg), **9** (2.0 mg), and **11** (48.0 mg). Subfraction 3-3-1-9 (111.0 mg) was separated on a Chromatorex ODS column (2 cm i.d. × 10 cm) with CH₃CN in H₂O (30%–100%, 10% stepwise elution), followed by preparative normal-phase HPLC to yield **10** (7.0 mg). Fraction 3-3-2 was separated

into five fractions on a Chromatorex ODS column (4 cm i.d. × 15 cm) with MeOH-H₂O (30–100%, 10% stepwise elution). Fraction 3-3-2-1 (137.3 mg) was separated by CC using silica gel with hexane-EtOAc (5:1) to give **11** (49.0 mg). Fraction 3-3-2-2 (528.1 mg) was separated on a silica gel column with hexane-EtOAc (10:1–8:1–6:1–4:1–0:1), followed by a Chromatorex ODS column to yield **5** (19.6 mg), and **6** (3.0 mg). Fraction 3-3-2-4 (2.3 g) was subjected to CC using silica gel with hexane-EtOAc (10:1–8:1–6:1–4:1–2:1–1:1) to yield **1** (1.0 g), and **2** (1.0 g).

7-Hydroxy-5-methoxyflavan (1)

Yellow amorphous power; $[\alpha]_{\text{D}}^{24}$ -24.8° ($c=0.99$, MeOH); ¹H-NMR (400 MHz, CD₃OD): δ 7.45 (2H, m, H-2', 6'), 7.36 (2H, m, H-3', 5'), 7.24 (1H, m, H-4'), 5.99 (1H, d, $J = 2.2$ Hz, H-6), 5.94 (1H, d, $J = 2.2$ Hz, H-8), 4.92 (1H, dd, $J = 2.2, 7.8$ Hz, H-2), 3.77 (3H, s, 5-OCH₃), 2.61 (2H, m, H-4), 2.81 (1H, m, H-3a), 1.90 (1H, m, H-3b); ¹³C-NMR (100 MHz, CD₃OD): δ 159.7 (C-5), 157.9 (C-7), 157.5 (C-8a), 143.4 (C-1'), 129.3 (C-2', 6'), 128.6 (C-4'), 126.9 (C-3',5'), 103.3 (C-4a), 96.8 (C-8), 92.5 (C-6), 78.8 (C-2), 55.8 (5-OCH₃), 30.9 (C-3), 20.1 (C-4).

7-Hydroxy-5-methoxy-6-methylflavan (2)

Yellow amorphous power; $[\alpha]_{\text{D}}^{24}$ -12.4° ($c=0.26$, MeOH); ¹H-NMR (400 MHz, CD₃OD): δ 7.38 (2H, m, H-2', 6'), 7.35 (2H, m, H-3', 5'), 7.33 (1H, m, H-4'), 6.14 (1H, s, H-8), 4.92 (1H, dd, $J = 2.2, 7.8$ Hz, H-2), 3.65 (3H, s, 5-OCH₃), 2.73 (2H, m, H-4), 2.12 (1H, m, H-3a), 1.93 (1H, m, H-3b), 2.09 (3H, s, 6-CH₃); ¹³C-NMR (100 MHz, CD₃OD): δ 158.5 (C-5), 156.1 (C-7), 155.1 (C-8a), 143.4 (C-1'), 129.4 (C-2', 6'), 128.6 (C-4'), 126.9 (C-3', 5'), 110.9 (C-6), 107.8 (C-4a), 100.0 (C-8), 78.8 (C-2), 60.2 (5-OCH₃), 31.1 (C-3), 20.6 (C-4), 8.7 (6-CH₃).

7,4'-Dihydroxy-8-methylflavan (3)

White amorphous power; $[\alpha]_{\text{D}}^{24}$ -1.3° ($c=0.25$, MeOH); ¹H-NMR (400 MHz, CDCl₃):

δ 7.23 (2H, d, $J = 8.4$ Hz, H-2', 6') 6.77 (2H, d, $J = 8.4$ Hz, H-3', 5') 6.72 (1H, d, $J = 8.0$ Hz, H-5), 6.31 (1H, d, $J = 8.0$ Hz, H-6), 4.93 (1H, dd, $J = 10.1, 2.5$ Hz, H-2), 2.84 (1H, m, H-4a), 2.66 (1H, m, H-4b), 2.08 (1H, m, H-3a), 1.90 (1H, m, H-3b), 2.05 (3H, s, 8-CH₃); ¹³C-NMR (100 MHz, CDCl₃): δ 155.0 (C-4'), 154.4 (C-8a), 153.6 (C-7), 135.7 (C-1'), 127.2 (C-2', 6'), 126.5 (C-5), 115.2 (C-3', 5'), 113.9 (C-4a), 111.4 (C-8), 107.2 (C-6), 77.2 (C-2), 30.0 (C-3), 24.8 (C-4), 8.1 (8-CH₃).

5,4'-Dihydroxy-7-methoxy-6-methylflavan (4)

White amorphous powder; $[\alpha]_D^{24} +0.12^\circ$ ($c=0.41$, MeOH); ¹H-NMR (400 MHz, CD₃OD): δ 7.22 (2H, d, $J = 8.5$ Hz, H-2', 6'), 6.77 (2H, d, $J = 8.5$ Hz, H-3', 5'), 6.03 (1H, s, H-8), 4.80 (1H, dd, $J = 10.1, 2.5$ Hz, H-2), 3.74 (3H, s, 7-OCH₃), 2.62 (1H, m, H-4a), 2.58 (1H, m, H-4b), 2.10 (1H, m, H-3a), 1.80 (1H, m, H-3b), 1.98 (3H, s, 6-CH₃); ¹³C-NMR (100 MHz, CD₃OD): δ 157.9 (C-4'), 157.1 (C-7), 155.5 (C-8a), 155.0 (C-5), 134.7 (C-1'), 128.2 (C-2', 6'), 116.0 (C-3', 5'), 104.9 (C-6), 103.2 (C-4a), 92.0 (C-8), 78.5 (C-2), 55.77 (7-OCH₃), 30.7 (C-3), 20.5 (C-4), 8.0 (8-CH₃).

7-Hydroxy-2,5-dimethoxy-6,8-dimethylflavan (5)

White amorphous powder; $[\alpha]_D^{24} 0^\circ$ ($c=0.41$, MeOH); EI-MS: m/z 314 [M]⁺, HR-EI-MS: m/z 314.1508 [M]⁺, (Calcd for C₁₉H₂₂O₄: 314.1519); IR cm⁻¹: 3452, 2938, 1608, 1469, 1125, 1042, 994, 927, 764, 700; UV λ_{\max} (MeOH) nm (log ϵ): 275 (3.03), 206 (4.54); ¹H NMR (500 MHz, CD₃OD): δ 7.58 (2H, dd, $J = 7.3, 1.4$ Hz, H-2', 6'), 7.41 (2H, t, $J = 7.3$ Hz, H-3', 5'), 7.34 (1H, tt, $J = 7.3, 1.4$ Hz, H-4'), 3.66 (3H, s, 5-OCH₃), 3.00 (3H, s, 2-OCH₃), 2.86 (1H, ddd, $J = 16.0, 12.6, 6.2$ Hz, H-4a), 2.68 (1H, ddd, $J = 16.0, 6.2, 2.3$ Hz, H-4b), 2.26 (1H, ddd, $J = 13.5, 6.2, 2.3$ Hz, H-3a), 2.18 (3H, s, 8-CH₃), 2.10 (3H, s, 6-CH₃), 1.74 (1H, ddd, $J = 13.5, 12.6, 6.2$ Hz, H-3b); ¹³C NMR (125 MHz, CD₃OD): δ 155.3 (C-5), 153.4 (C-7), 149.7 (C-8a), 142.9 (C-1'), 129.4 (C-3', 5'), 129.2 (C-4'), 127.3 (C-2', 6'), 111.8 (C-6), 109.9 (C-8), 108.8 (C-4a), 101.1 (C-2), 60.4 (5-OCH₃), 50.2 (2-OCH₃), 35.1 (C-3), 17.7 (C-4), 9.3 (6-CH₃), 8.6

(8-CH₃).

7-Hydroxy-2,5-dimethoxy-8-methylflavan (6)

White amorphous powder; $[\alpha]_D^{24}$ 0° (c=0.14, MeOH); EI-MS: m/z 300 [M]⁺, HR-EI-MS: m/z 300.1347 [M]⁺, (Calcd for C₁₈H₂₀O₄: 300.1362); IR cm⁻¹: 3399, 2934, 1617, 1446, 1150, 1095, 994, 927, 762, 700; UV λ_{max} (MeOH) nm (log ϵ): 272 (3.23), 209 (4.57); ¹H NMR (500 MHz, CD₃OD): δ 7.58 (2H, dd, J = 7.3, 1.4 Hz, H-2', 6'), 7.41 (2H, t, J = 7.3 Hz, H-3', 5'), 7.34 (1H, tt, J = 7.3, 1.4 Hz, H-4'), 6.11 (1H, s, H-6), 3.74 (3H, s, 5-OCH₃), 2.99 (3H, s, 2-OCH₃), 2.67 (1H, ddd, J = 16.3, 12.4, 6.4 Hz, H-4a), 2.57 (1H, ddd, J = 16.3, 6.4, 2.5 Hz, H-4b), 2.23 (1H, ddd, J = 13.5, 6.4, 2.5 Hz, H-3a), 2.11 (3H, s, 8-CH₃), 1.74 (1H, ddd, J = 13.5, 12.4, 6.4 Hz, H-3b); ¹³C NMR (125 MHz, CD₃OD): δ 156.8 (C-5), 155.2 (C-7), 152.1 (C-8a), 143.0 (C-1'), 129.4 (C-3', 5'), 129.2 (C-4'), 127.3 (C-2', 6'), 105.3 (C-8), 103.6 (C-4a), 101.2 (C-2), 92.7 (C-6), 55.7 (5-OCH₃), 50.2 (2-OCH₃), 34.9 (C-3), 17.3 (C-4), 8.0 (8-CH₃).

7-Hydroxy-2,5-dimethoxy-6-methylflavan (7)

White amorphous powder; $[\alpha]_D^{24}$ 0° (c=0.25, MeOH); EI-MS: m/z 300 [M]⁺, HR-EI-MS: m/z 300.1359 [M]⁺, (Calcd for C₁₈H₂₀O₄: 300.1362); IR cm⁻¹: 3388, 2938, 1619, 1448, 1148, 1045, 993, 915, 763, 700; UV λ_{max} (MeOH) nm (log ϵ): 281 (3.44), 205 (4.67); ¹H NMR (500 MHz, CD₃OD): δ 7.53 (2H, dd, J = 7.3, 1.4 Hz, H-2', 6'), 7.38 (2H, t, J = 7.3 Hz, H-3', 5'), 7.32 (1H, tt, J = 7.3, 1.4 Hz, H-4'), 6.29 (1H, s, H-8), 3.67 (3H, s, 5-OCH₃), 3.02 (3H, s, 2-OCH₃), 2.83 (1H, ddd, J = 16.0, 12.6, 6.2 Hz, H-4a), 2.64 (1H, ddd, J = 16.0, 6.2, 2.3 Hz, H-4b), 2.21 (1H, ddd, J = 13.5, 6.2, 2.3 Hz, H-3a), 2.06 (3H, s, 6-CH₃), 1.72 (1H, ddd, J = 13.5, 12.6, 6.2 Hz, H-3b); ¹³C NMR (125 MHz, CD₃OD): δ 158.1 (C-5), 156.2 (C-7), 152.3 (C-8a), 142.7 (C-1'), 129.3 (C-3', 5'), 129.2 (C-4'), 127.3 (C-2', 6'), 111.6 (C-6), 108.4 (C-4a), 101.2 (C-2), 100.5 (C-8), 60.3 (5-OCH₃), 50.4 (2-OCH₃), 35.4 (C-3), 17.6 (C-4), 8.8 (6-CH₃).

7,4'-Dihydroxy-8-methoxyhomoisoflavane (8)

White amorphous powder; $[\alpha]_{\text{D}}^{21} +48.3^\circ$ ($c=0.22$, MeOH); $^1\text{H-NMR}$ (400 MHz, CDCl_3): δ 7.98 (2H, d, $J = 8.3$ Hz, H-2', 6'), 6.71 (2H, d, $J = 8.3$ Hz, H-3', 5'), 6.56 (1H, d, $J = 8.2$ Hz, H-5), 6.43 (1H, d, $J = 8.2$ Hz, H-8), 4.18 (1H, m, H-2a), 3.81 (3H, s, -OCH₃), 3.78 (1H, m, H-2b), 2.68, (1H, dd, $J = 15.9, 4.6$ Hz, H-4a), 2.57 (1H, dd, $J = 13.7, 7.6$ Hz, H-9a), 2.51 (1H, dd, $J = 13.7, 7.6$ Hz, H-9b), 2.39 (1H, dd, $J = 15.9, 8.6$ Hz, H-4b), 2.22 (1H, m, H-3). $^{13}\text{C-NMR}$ (100 MHz, CDCl_3): δ 154.0 (C-4'), 147.4 (C-7), 147.2 (C-8a), 134.6 (C-8), 131.4 (C-1'), 130.0 (C-2', 6'), 124.4 (C-5), 115.2 (C-3', 5'), 114.4 (C-4a), 107.0 (C-6), 70.0 (C-2), 60.8 (-OCH₃), 37.0 (C-9), 34.0 (C-3), 30.4 (C-4).

6,4'-Dihydroxy-7-methoxyhomoisoflavan (9)

White amorphous powder; $[\alpha]_{\text{D}}^{21} +71.6^\circ$ ($c=0.17$, MeOH); $^1\text{H-NMR}$ (400 MHz, CDCl_3): δ 7.03 (2H, d, $J = 8.2$ Hz, H-2', 6'), 6.84 (2H, d, $J = 8.2$ Hz, H-3', 5'), 6.44 (1H, s, H-5), 6.40 (1H, s, H-8), 4.10 (1H, m, H-2a), 3.78 (3H, s, -OCH₃), 3.72 (1H, m, H-2b), 2.64, (1H, m, H-4a), 2.59 (1H, m, H-9a), 2.52 (1H, m, H-9b) 2.42 (1H, m, H-4b), 2.22 (1H, m, H-3).

4,4'-Dihydroxy-2,6-dimethoxy-dihydrochalcone (10)

White amorphous powder; $^1\text{H-NMR}$ (400 MHz, CD_3OD): δ 7.76 (2H, d, $J = 8.0$ Hz, H-2', 6'), 6.72 (2H, d, $J = 8.0$ Hz, H-3', 5'), 5.96 (2H, s, H-3, H-5), 3.79 (6H, s, -OCH₃), 2.80 (4H, m, H- α , H- β); $^{13}\text{C-NMR}$ (100 MHz, CD_3OD): δ 202.5 (C=O), 163.6 (C-4'), 160.1 (C-2, C-6), 158.5 (C-4), 132.0 (C-2', 6'), 130.0 (C-1'), 116.1 (C-3', 5'), 109.1 (C-1), 92.7 (C-3, C-5), 55.9 (-OCH₃), 39.6 (C- α), 20.2 (C- β).

4,6-Dihydroxy-3-methyl-2-methoxychalcone (11)

Yellow amorphous powder; $^1\text{H-NMR}$ (400 MHz, CD_3OD): δ 8.01 (2H, m, H-2', 6'), 7.54 (1H, m, H-4'), 7.45 (2H, m, H-3', 5'), 6.20 (1H, s, H-5), 3.65 (3H, s, 2-OCH₃),

3.17 (2H, t, $J = 8.3$ Hz, H-8), 2.88 (2H, t, $J = 8.3$ Hz, H-7), 2.01 (3H, s, 3-CH₃);
¹³C-NMR (100 MHz, CD₃OD): δ 203.1 (C-9), 159.3 (C-2), 156.0 (C-4), 155.2 (C-6),
138.1 (C-1'), 134.2 (C-4'), 129.6 (C-2', 6'), 129.3 (C-3', 5'), 113.0 (C-1), 109.8 (C-3),
99.9 (C-5), 61.2 (2-OCH₃), 40.4 (C-8), 20.5 (C-7), 8.9 (3-CH₃).

Chapter 3

Extraction and Isolation

The resin (450 g, purchased at a local market in Yunnan Province, China, in 2012) of *D. cochinchinensis* was dissolved in MeOH and any insoluble materials were removed by filtration. The MeOH soluble part (432 g) was partitioned between hexane and MeOH. The MeOH layer (414.89 g) was separated by Sephadex LH-20 CC (40 × 10 cm, i.d., 100–60% EtOH, 50% acetone) to give five fractions, Fr. 1 (20.8 g), Fr. 2 (292.0 g), Fr. 3 (70.1 g), Fr. 4 (16.3 g) and Fr. 5 (14.2 g). Fr. 2 was suspended in CHCl₃ to give a CHCl₃ soluble part (Fr. 2-1, 64.0 g) and insolubles (Fr. 2-2, 228.0 g). The CHCl₃ soluble part was separated by silica gel CC (20 × 8 cm, i.d., CHCl₃-MeOH 100:0–0:100, v/v) into five fractions, Frs. 2-1-1 (18.5 g), 2-1-2 (1.24 g), 2-1-3 (8.9 g), 2-1-4 (8.9 g) and 2-1-5 (18.2 g). Fr. 2-1-2 was subjected to further purification by successive rounds of CC over Chromatorex ODS (H₂O-MeOH), silica gel (hexane-EtOAc) and preparative HPLC to give **3** (89.9 mg), **4** (69.8 mg), **12** (68.8 mg), **16** (7.1 mg) and **24** (18.0 mg). The purification of Fr. 2-1-4 by successive rounds of CC over MCI gel (H₂O-MeOH), Sephadex LH-20 (H₂O-MeOH), silica gel (hexane-EtOAc), Chromatorex ODS (H₂O-MeOH) and preparative HPLC gave **10** (22.1 mg), **19** (49.9 mg), **20** (28.8 mg), **21** (30.1 mg), **25** (19.1 mg), **26** (6.7 mg), **27** (9.0 mg), **28** (16.8 mg), **30** (5.7 mg), **31** (7.3 mg), **32** (7.5 mg) and **33** (7.6 mg). A portion (145.0 g) of the CHCl₃ insoluble part (Fr. 2-2) was purified by CC over a Sephadex LH-20 column (40 × 10 cm, i.d., 60–100% MeOH, 60–80% acetone) to give five fractions. Fr. 2-2-1 (26.0 g) and Fr. 2-2-2 (37.1 g) contained steroids, and Fr. 2-2-3 (65.6 g), Fr. 2-2-4 (14.3 g) and Fr. 2-2-5 (0.6 g) contained phenolic substances.

Fr. 2-2-1 was purified by CC over silica gel eluting with CHCl_3 -MeOH- H_2O , followed by purification of Chromatorex ODS to yield **34** (36.7 mg) and **35** (9.8 mg). Fr. 2-2-3 was purified over a Diaion HP 20SS column (35×7 cm, i.d., 60–100% MeOH, acetone) to give 12 subfractions. Subfraction 2-2-3-1 (1.45 g) was purified by CC over silica gel (3 cm i.d. \times 15 cm) with CHCl_3 -MeOH (20:1–4:1, v/v) to yield **22** (660.8 mg). Subfraction 2-2-3-2 (3.72 g) was repeatedly chromatographed over Sephadex LH-20, silica gel and Chromatorex ODS to yield **13** (16.6 mg), **14** (136.6 mg), **18** (6.1 mg) and **29** (126.4 mg). Subfraction 2-2-3-3 (5.31 g) was purified by sequential CC over Sephadex LH-20, silica gel and Chromatorex ODS columns to yield **15** (162.8 mg), **17** (53.8 mg) and **23** (49.7 mg).

Cochinchinenene G (12)

Yellow amorphous powder; $[\alpha]_{\text{D}}^{21} -1.6^\circ$ ($c=0.55$, MeOH), FAB-MS m/z : 527 $[\text{M}+\text{H}]^+$; HR-FAB-MS m/z : 527.2437 $[\text{M}+\text{H}]^+$ (Calcd for $\text{C}_{33}\text{H}_{35}\text{O}_6$: 527.2434); IR ν_{max} cm^{-1} : 3388, 2999, 2937, 2834, 1599, 1511, 1462; UV λ_{max} (MeOH) nm (log ϵ): 299 (1.98), 287 (2.04), 218 (3.50); ^1H - and ^{13}C -NMR data see Table 2.

Cochinchinenene H (13)

Yellow amorphous powder; $[\alpha]_{\text{D}}^{21} -0.7^\circ$ ($c=0.15$, MeOH); FAB-MS m/z : 485 $[\text{M}+\text{H}]^+$; HR-FAB-MS m/z : 485.1961 $[\text{M}+\text{H}]^+$ (Calcd for $\text{C}_{30}\text{H}_{29}\text{O}_6$: 485.1964); IR ν_{max} cm^{-1} : 3360, 2927, 1603, 1509, 1430, 1232, 1195, 1172; UV λ_{max} (MeOH) nm (log ϵ): 325 (1.78), 216 (2.42); ^1H - and ^{13}C -NMR data see Table 2.

Cochinchinenene D (14)

White amorphous powder; $[\alpha]_{\text{D}}^{21} +6.0^\circ$ ($c=0.21$, MeOH), ESI-MS m/z : 483 $[\text{M}-\text{H}]^-$; ^1H -NMR (500 MHz, CD_3OD): δ 6.25 (1H, d, $J = 2.5$ Hz, H-4), 6.49 (1H, d, $J = 2.5$ Hz, H-6), 7.13 (2H, d, $J = 8.5$ Hz, H-2', 6'), 6.74 (2H, d, $J = 8.5$ Hz, H-3', 5'), 6.94 (1H, d, $J = 16.0$ Hz, H- α), 6.65 (1H, d, $J = 16.0$ Hz, H- β), 6.36 (1H, d, $J = 2.5$ Hz, H-3''),

6.23 (1H, dd, $J = 8.0, 2.5$ Hz, H-5''), 6.77 (1H, d, $J = 8.0$ Hz, H-6''), 7.15 (2H, d, $J = 8.5$ Hz, H-2''', 6'''), 6.76 (2H, d, $J = 8.5$ Hz, H-3''', 5'''), 4.62 (1H, m, -CH), 2.35 (2H, m, H- α'), 2.51 (2H, m, H- β'), 3.63 (3H, s, 2''-OCH₃); ¹³C-NMR (125 MHz, CD₃OD): δ 140.7 (C-1), 122.6 (C-2), 158.0 (C-3), 102.9 (C-4), 156.7 (C-5), 105.7 (C-6), 130.9 (C-1'), 128.6 (C-2', 6'), 116.3 (C-3', 5'), 158.5 (C-4'), 126.9 (C- α), 130.6 (C- β), 123.3 (C-1''), 159.7 (C-2''), 99.6 (C-3''), 157.3 (C-4''), 107.4 (C-5''), 131.2 (C-6''), 138.0 (C-1'''), 129.7 (C-2''', 6'''), 115.5 (C-3''', 5'''), 156.2 (C-4'''), 41.0 (-CH), 34.7 (C- α'), 29.2 (C- β'), 56.1 (2''-OCH₃).

Cochinchinenene C (15)

White amorphous powder; $[\alpha]_{\text{D}}^{21} +1.2^\circ$ ($c=0.32$, MeOH), ESI-MS m/z : 497 [M-H]⁻; ¹H-NMR (500 MHz, CD₃OD): δ 6.35 (1H, d, $J = 2.4$ Hz, H-4), 6.57 (1H, d, $J = 2.4$ Hz, H-6), 7.14 (2H, d, $J = 8.0$ Hz, H-2', 6'), 6.75 (2H, d, $J = 8.0$ Hz, H-3', 5'), 6.95 (1H, d, $J = 16.0$ Hz, H- α), 6.67 (1H, d, $J = 16.0$ Hz, H- β), 6.33 (1H, d, $J = 2.4$ Hz, H-3''), 6.22 (1H, dd, $J = 8.2, 2.4$ Hz, H-5''), 6.72 (1H, d, $J = 8.2$ Hz, H-6''), 7.07 (2H, d, $J = 8.5$ Hz, H-2''', 6'''), 6.66 (2H, d, $J = 8.5$ Hz, H-3''', 5'''), 4.19 (1H, m, -CH), 2.37 (2H, m, H- α'), 2.43 (2H, m, H- β'), 3.67 (3H, s, 3-OCH₃), 3.62 (3H, s, 2''-OCH₃); ¹³C-NMR (125 MHz, CD₃OD): δ 140.5 (C-1), 124.2 (C-2), 160.6 (C-3), 99.7 (C-4), 157.8 (C-5), 106.3 (C-6), 131.1 (C-1'), 129.7 (C-2', 6'), 116.4 (C-3', 5'), 158.1 (C-4'), 128.7 (C- α), 131.7 (C- β), 123.0 (C-1''), 159.7 (C-2''), 99.7 (C-3''), 157.5 (C-4''), 107.5 (C-5''), 131.7 (C-6''), 137.7 (C-1'''), 130.6 (C-2''', 6'''), 116.0 (C-3''', 5'''), 155.6 (C-4'''), 41.0 (-CH), 34.7 (C- α'), 29.8 (C- β'), 56.4 (3-OCH₃), 56.1 (2''-OCH₃).

Cochinchinenene A (16)

Yellow amorphous powder; $[\alpha]_{\text{D}}^{21} +6.5^\circ$ ($c=0.17$, MeOH), ¹H-NMR (400 MHz, CD₃OD): δ 7.36 (2H, d, $J = 8.3$ Hz, H-2', 6'), 7.19 (2H, d, $J = 8.8$ Hz, H-2''', 6'''), 7.02 (1H, d, $J = 16.1$ Hz, H- β), 6.89 (1H, d, $J = 16.1$ Hz, H- α), 6.77 (2H, d, $J = 8.3$ Hz, H-3', 5'), 6.74 (1H, d, $J = 8.1$ Hz, H-6''), 6.73 (2H, s, H-2, 6), 6.71 (2H, d, $J = 8.8$ Hz,

H-3''', 5'''), 6.34 (1H, d, $J = 2.2$ Hz, H-3''), 6.25 (1H, dd, $J = 8.1, 2.2$ Hz, H-5''), 4.56 (1H, t, $J = 7.7$ Hz, -CH), 3.76 (6H, s, 3, 5-OCH₃), 3.71 (6H, s, 2'', 4'''-OCH₃), 2.40 (2H, m, H-β'), 2.30 (2H, m, H-α'); ¹³C-NMR (100 MHz, CD₃OD): δ 160.1 (C-3, 5), 159.6 (C-2''), 158.7 (C-4'''), 158.3 (C-4'), 157.5 (C-4''), 138.9 (C-1'''), 138.6 (C-1), 131.1 (C-6''), 130.5 (C-1'), 130.0 (C-2''', 6'''), 129.0 (C-β), 128.8 (C-2', 6'), 127.1 (C-α), 123.3 (C-1''), 122.1 (C-4), 116.4 (C-3', 5'), 113.9 (C-3'', 5''), 107.4 (C-5''), 103.7 (C-2, C-6), 99.7 (C-3''), 56.1 (3-OCH₃, 5-OCH₃), 55.6 (2''-OCH₃), 55.5 (4'''-OCH₃), 40.2 (-CH), 34.0 (C-α'), 29.7 (C-β').

1-[5-(4,4'-Dihydroxy-2-methoxydihydrochalconyl)]-1-(4-hydroxyphenyl)-3-(4-hydroxy-2-methoxyphenyl)propane (17)

Yellow amorphous powder; $[\alpha]_{\text{D}}^{21} +4.0^{\circ}$ ($c=0.23$, MeOH), ESI-MS m/z : 527 [M-H]⁻; ¹H-NMR (500 MHz, CD₃OD): δ 6.34 (1H, s, H-3), 6.87 (1H, s, H-6), 7.81 (2H, d, $J = 8.6$ Hz, H-2', 6'), 6.78 (2H, d, $J = 8.6$ Hz, H-3', 5'), 3.08 (2H, t, $J = 7.6$ Hz, H-α), 2.84 (2H, t, $J = 7.6$ Hz, H-β), 6.34 (1H, overlap, H-3''), 6.26 (1H, dd, $J = 8.0, 2.4$ Hz, H-5''), 6.77 (1H, d, $J = 8.0$ Hz, H-6''), 7.02 (2H, d, $J = 8.6$ Hz, H-2''', 6'''), 6.64 (2H, d, $J = 8.6$ Hz, H-3''', 5'''), 4.15 (1H, t, $J = 7.8$ Hz, -CH), 2.09 (2H, m, H-α'), 2.38 (2H, m, H-β'), 3.71 (6H, s, 2, 2''-OCH₃); ¹³C-NMR (125 MHz, CD₃OD): δ 121.0 (C-1), 157.2 (C-2), 99.7 (C-3), 155.1 (C-4), 124.7 (C-5), 130.1 (C-6), 130.0 (C-1'), 132.0 (C-2', 6'), 116.1 (C-3', 5'), 163.6 (C-4'), 202.1 (C=O), 123.2 (C-1''), 159.6 (C-2''), 99.8 (C-3''), 157.5 (C-4''), 107.5 (C-5''), 131.1 (C-6''), 138.3 (C-1'''), 129.9 (C-2''', 6'''), 115.7 (C-3''', 5'''), 155.9 (C-4'''), 43.0 (-CH), 40.0 (C-α), 27.3 (C-β), 37.2 (C-α'), 29.5 (C-β'), 55.6 (2-OCH₃), 55.7 (2''-OCH₃).

Cochinchinenin (18)

Yellow amorphous powder; $[\alpha]_{\text{D}}^{21} +2.1^{\circ}$ ($c=0.18$, MeOH), ESI-MS m/z : 513 [M-H]⁻; ¹H-NMR (500 MHz, CD₃OD): δ 6.26 (1H, s, H-3), 6.82 (1H, s, H-6), 7.86 (2H, d, $J = 8.9$ Hz, H-2', 6'), 6.79 (2H, d, $J = 8.9$ Hz, H-3', 5'), 3.13 (2H, t, $J = 7.7$ Hz, H-α), 2.86

(2H, t, $J = 7.7$ Hz, H- β), 6.34 (1H, d, $J = 2.2$ Hz, H-3''), 6.25 (1H, dd, $J = 8.2, 2.2$ Hz, H-5''), 6.77 (1H, d, $J = 8.2$ Hz, H-6''), 7.01 (2H, d, $J = 8.2$ Hz, H-2''', 6'''), 6.63 (2H, d, $J = 8.2$ Hz, H-3''', 5'''), 4.10 (1H, t, $J = 8.0$ Hz, -CH), 2.03 (2H, m, H- α'), 2.36 (2H, m, H- β'), 3.72 (3H, s, 2''-OCH₃); ¹³C-NMR (125 MHz, CD₃OD): δ 119.4 (C-1), 154.5 (C-2), 103.6 (C-3), 155.8 (C-4), 124.5 (C-5), 130.1 (C-6), 130.0 (C-1'), 132.0 (C-2', 6'), 116.1 (C-3', 5'), 163.7 (C-4'), 202.3 (C=O), 123.2 (C-1''), 159.6 (C-2''), 99.7 (C-3''), 157.5 (C-4''), 107.5 (C-5''), 131.1 (C-6''), 138.5 (C-1'''), 129.9 (C-2''', 6'''), 115.6 (C-3''', 5'''), 157.5 (C-4'''), 43.1 (-CH), 40.0 (C- α), 27.1 (C- β), 37.3 (C- α'), 29.5 (C- β'), 55.6 (2''-OCH₃).

Loureirin C (19)

Colorless crystal; ¹H-NMR (400 MHz, CD₃OD): δ 7.84 (2H, d, $J = 8.78$ Hz, H-2', 6'), 6.90 (1H, d, $J = 8.05$ Hz, H-6), 6.82 (2H, d, $J = 8.78$ Hz, H-3', 5'), 6.37 (1H, d, $J = 2.19$ Hz, H-5), 6.27 (1H, dd, $J = 8.05, 2.19$ Hz, H-3), 3.07 (2H, t, $J = 7.5$, H- β), 2.82 (2H, t, $J = 7.5$, H- α); ¹³C-NMR (100 MHz, CD₃OD): δ 201.7 (C=O), 163.6 (C-4'), 159.6 (C-2), 158.1 (C-4), 131.8 (C-2',6'), 131.2 (C-6), 129.9 (C-1), 121.3 (C-1'), 116.1 (C-3',5'), 107.6 (C-5), 99.7 (C-3), 55.5 (-OCH₃), 39.9 (C- β), 26.8 (C- α).

4,4'-Dihydroxy-2'-methoxychalcone (20)

Yellow needles; ¹H-NMR (400 MHz, CD₃OD): δ 8.05 (1H, d, $J = 15.6$ Hz, H- β), 7.96 (2H, d, $J = 8.8$ Hz, H-2', 6'), 7.62 (1H, d, $J = 15.6$ Hz, H- α), 7.61 (1H, d, $J = 8.4$ Hz, H-6), 6.88 (2H, d, $J = 8.8$ Hz, H-3', 5'), 6.46 (1H, dd, $J = 8.4, 2.0$ Hz, H-5), 6.43 (1H, d, $J = 2.0$ Hz, H-3), 3.88 (3H, s, -OCH₃); ¹³C-NMR (100 MHz, CD₃OD): δ 191.6 (C=O), 163.5 (C-4'), 163.1 (C-4), 163.1 (C-2), 141.3 (C- β), 132.1 (C-2', 6'), 131.6 (C-6), 131.4 (C-1'), 119.4 (C- α), 116.7 (C-1), 116.3 (C-3', 5'), 109.2 (C-5), 99.9 (C-3), 56.0 (-OCH₃).

4,4'-dihydroxy-3'-methoxychalcone (21)

Yellow needles; $^1\text{H-NMR}$ (400 MHz, CD_3OD): δ 7.58 (1H, d, $J = 15.6$ Hz, H- β), 7.55 (1H, d, $J = 8.3$ Hz, H-3), 7.50 (2H, d, $J = 8.5$ Hz, H-2', 6'), 7.42 (1H, d, $J = 15.6$ Hz, H- α), 6.82 (2H, d, $J = 8.5$ Hz, H-3', 5'), 6.50 (1H, s, H-6), 6.46 (1H, d, $J = 8.3$ Hz, H-2), 3.87 (3H, s, $-\text{OCH}_3$).

Resveratrol (22)

White needles; $^1\text{H-NMR}$ (400 MHz, CD_3OD): δ 7.34 (2H, d, $J = 8.8$ Hz, H-2', 6'), 6.96 (1H, d, $J = 16.4$ Hz, H- β), 6.80 (1H, d, $J = 16.4$ Hz, H- α), 6.74 (2H, d, $J = 8.8$ Hz, H-3', 5'), 6.44 (2H, d, $J = 2.0$ Hz, H-2, 6), 6.16 (1H, d, $J = 2.0$ Hz, H-4); $^{13}\text{C-NMR}$ (100 MHz, CD_3OD): δ 159.6 (C-3), 158.3 (C-4'), 141.3 (C-1'), 130.4 (C-1), 129.3 (C- β), 128.7 (C-2', 6'), 126.9 (C- α), 116.4 (C-3', 5'), 105.7 (C-2, 6), 102.6 (C-4).

3-O-methyl resveratrol (23)

Colorless crystal; $^1\text{H-NMR}$ (400 MHz, CD_3OD): δ 7.31 (2H, d, $J = 8.4$ Hz, H-2', 6'), 6.96 (1H, d, $J = 16.4$ Hz, H- β), 6.80 (1H, d, $J = 16.4$ Hz, H- α), 6.73 (2H, d, $J = 8.4$ Hz, H-3', 5'), 6.51 (2H, d, $J = 2.2$ Hz, H-2, 6), 6.20 (1H, d, $J = 2.2$ Hz, H-4), 3.71 (3H, s, $-\text{OCH}_3$).

7,4'-Homoisoflavane (24)

White crystal; $[\alpha]_{\text{D}}^{21} +64.0^\circ$ ($c=0.26$, MeOH), $^1\text{H-NMR}$ (400 MHz, CD_3OD): δ 7.00 (2H, d, $J = 8.0$ Hz, H-2', 6'), 6.76 (1H, d, $J = 8.0$ Hz, H-5), 6.72 (2H, d, $J = 8.0$ Hz, H-3', 5'), 6.27 (1H, d, $J = 8.0$ Hz, H-6), 6.17 (1H, s, H-8), 4.06 (1H, m, H-2a), 3.15 (1H, m, H-2b), 2.65 (1H, m, H-9a), 2.49 (2H, m, H-4), 2.36 (1H, m, H-9b), 2.11 (1H, m, H-3); $^{13}\text{C-NMR}$ (100 MHz, CD_3OD): δ 157.8 (C-7), 156.7 (C-8a), 156.5 (C-4'), 131.7 (C-1'), 131.3 (C-5), 130.9 (C-2', 6'), 116.1 (C-3', 5'), 113.8 (C-4a), 109.0 (C-6), 103.7 (C-8), 70.9 (C-2), 38.0 (C-9), 35.9 (C-3), 31.3 (C-4).

10,11-Dihydroxydracaenone C (25)

Colorless crystal; ¹H-NMR (400 MHz, DMSO-*d*₆): δ 6.81 (1H, d, *J* = 9.76 Hz, H-1), 6.55 (1H, s, H-9), 6.33 (1H, dd, *J* = 9.76, 1.70 Hz, H-2), 6.30 (1H, s, H-12), 5.40 (1H, d, *J* = 1.70 Hz, H-4), 4.31 (1H, d, *J* = 10.9 Hz, H-6), 4.13 (1H, dd, *J* = 10.9, 2.68 Hz, H-6), 3.13 (1H, dd, *J* = 17.08, 6.43 Hz, H-8), 2.85 (1H, d, *J* = 17.08 Hz, H-8), 2.36 (1H, s, H-7), 2.14 (1H, d, *J* = 12.45 Hz, H-13), 1.99 (1H, dd, *J* = 12.45, 2.68 Hz, H-13); ¹³C-NMR (100 MHz, DMSO-*d*₆): δ 187.7 (C-3), 177.9 (C-4a), 150.2 (C-1), 145.1 (C-10), 143.3 (C-11), 128.6 (C-2), 126.8 (C-12a), 125.0 (C-8a), 115.9 (C-9), 112.7 (C-12), 107.0 (C-4), 77.0 (C-6), 42.0 (C-12b), 33.7 (C-8), 31.9 (C-13), 28.0 (C-7).

7,4'-Dihydroxyflavanone (26)

Yellow amorphous powder; [α]_D²¹ -9.0° (*c*=0.13, MeOH), ¹H-NMR (400 MHz, CD₃OD): δ 7.73 (1H, d, *J* = 8.78 Hz, H-5), 7.32 (2H, d, *J* = 8.54 Hz, H-2', 6'), 6.81 (2H, d, *J* = 8.54 Hz, H-3', 5'), 6.49 (1H, dd, *J* = 8.78, 2.19 Hz, H-6), 6.34 (1H, d, *J* = 2.19 Hz, H-8), 5.38 (1H, d, *J* = 13.18 Hz, H-2), 3.08 (1H, t, *J* = 16.60 Hz, H-3a), 2.69 (1H, d, *J* = 16.80 Hz, H-3a); ¹³C-NMR (100 MHz, CD₃OD): δ 193.5 (C-4), 166.7 (C-7), 165.5 (C-8a), 158.9 (C-4'), 131.3 (C-1'), 129.8 (C-5), 129.0 (C-2', 6'), 116.3 (C-3', 5'), 114.9 (C-4a), 111.7 (C-6), 103.8 (C-8), 81.0 (C-2), 44.9 (C-3).

7,4'-Dihydroxyhomoisoflavanone (27)

White amorphous powder; [α]_D²¹ -20.2° (*c*=0.21, MeOH), ¹H-NMR (400 MHz, CD₃OD): δ 7.63 (1H, d, *J* = 8.78 Hz, H-5), 6.95 (2H, d, *J* = 8.54 Hz, H-2', 6'), 6.64 (2H, d, *J* = 8.54 Hz, H-3', 5'), 6.40 (1H, dd, *J* = 8.78, 2.19 Hz, H-6), 6.20 (1H, d, *J* = 2.19 Hz, H-8), 4.21 (1H, dd, *J* = 11.47, 4.15 Hz, H-2), 4.07 (1H, dd, *J* = 11.47, 7.32 Hz, H-2), 2.98 (1H, dd, *J* = 13.80, 4.63 Hz, H-9), 2.61 (1H, m, H-3), 2.15 (1H, dd, *J* = 13.80, 4.63 Hz, H-9); ¹³C-NMR (100 MHz, CD₃OD): δ 195.0 (C-4), 166.5 (C-7), 165.3 (C-4'), 157.1 (C-8a), 131.1 (C-2', 6'), 130.4 (C-1'), 130.2 (C-5), 116.3 (C-3', 5'), 114.5 (C-4a), 111.7 (C-6), 103.4 (C-8), 70.7 (C-2), 48.9 (C-3), 33.0 (C-9).

3',7-Dihydroxy-4'-methoxyflavone (28)

Yellow amorphous powder; ¹H-NMR (400 MHz, CD₃OD): δ 7.97 (1H, d, *J* = 8.8 Hz, H-5), 7.54 (1H, dd, *J* = 8.0, 2.4 Hz, H-6'), 7.51 (1H, d, *J* = 2.4 Hz, H-2'), 6.98 (1H, d, *J* = 2.0 Hz, H-8), 6.95 (1H, d, *J* = 8.0 Hz, H-5'), 6.93 (1H, dd, *J* = 8.8, 2.0 Hz, H-6), 6.70 (1H, s, H-3), 3.69 (3H, s, -OCH₃).

7,4'-Dihydroxyflavone (29)

Yellow amorphous powder; ¹H-NMR (400 MHz, CD₃OD): δ 7.91 (2H, d, *J* = 8.8 Hz, H-2', 6'), 7.85 (1H, d, *J* = 8.4 Hz, H-5), 6.95 (1H, d, *J* = 2.0 Hz, H-8), 6.92 (2H, d, *J* = 8.8 Hz, H-3', 5'), 6.88 (1H, d, *J* = 2.0 Hz, H-6), 6.71 (1H, s, H-3); ¹³C-NMR (100 MHz, CD₃OD): δ 176.2 (C-4), 162.5 (C-7), 162.4 (C-2), 160.6 (C-8a), 157.3 (C-4'), 128.0 (C-2', 6'), 126.4 (C-5), 121.7 (C-1'), 116.0 (C-4a), 115.8 (C-3', 5'), 114.7 (C-6), 104.4 (C-3), 102.4 (C-8).

(-)-(7'S,8S,8'R)-7',9-epoxy-4,4',9'-trihydroxy-3,3',5,5'-tetramethoxylignan-7-one (30)

White amorphous powder; [α]_D²¹ -24.4° (*c*=0.42, MeOH), ¹H-NMR (400 MHz, CD₃OD): δ 7.38 (2H, s, H-2, 6), 6.71 (2H, s, H-2', 6'), 4.62 (1H, d, *J* = 8.3 Hz, H-7'), 4.24 (1H, m, H-8), 4.22 (2H, m, H-9), 3.69 (1H, dd, *J* = 11.4, 4.6 Hz, H-9'a), 3.63 (1H, dd, *J* = 11.4, 4.6 Hz, H-9'b), 2.64 (1H, m, H-8'), 3.91 (6H, s, 3, 5-OCH₃), 3.84 (6H, s, 3', 5'-OCH₃); ¹³C-NMR (100 MHz, CD₃OD): δ 200.3 (C-7), 149.2 (C-3, 5), 149.1 (C-3', 5'), 142.9 (C-4), 136.3 (C-4'), 132.9 (C-1'), 128.6 (C-1), 107.7 (C-2, 6), 105.2 (C-2', 6'), 85.4 (C-7'), 71.5 (C-9), 61.3 (C-9'), 56.9(3, 5-OCH₃), 56.7 (3', 5'-OCH₃), 55.1 (C-8'), 50.1 (C-8).

Secoisolariciresinol (31)

White amorphous powder; [α]_D²¹ -23.5° (*c*=0.42, MeOH), ¹H-NMR (400 MHz, CD₃OD): δ 6.65 (2H, d, *J* = 7.81 Hz, H-2, 2'), 6.57 (2H, d, *J* = 1.95 Hz, H-1, 1'), 6.52

(2H, d, $J = 7.81$ Hz, H-5, 5'), 3.57 (2H, m, H-9, 9'), 2.66 (2H, dd, $J = 13.6, 6.8$ Hz, H-7, 7'), 1.89 (2H, s, H-8, 8'), 3.72 (6H, s, -OCH₃); ¹³C-NMR (100 MHz, CD₃OD): δ 148.8 (C-4, 4'), 145.4 (C-3, 3'), 133.8 (C-1, 1'), 122.7 (C-6, 6'), 115.7 (C-2, 2'), 113.3 (C-5, 5'), 62.1 (C-9, 9'), 56.1 (-OCH₃), 44.1 (C-8, 8'), 36.0 (C-7, 7').

Dihydrodehydroconiferyl alcohol (32)

White amorphous powder; $[\alpha]_{D}^{21} +9.4^{\circ}$ ($c=0.26$, MeOH), ¹H-NMR (400 MHz, CD₃OD): δ 6.72 (2H, s, H-2', 6'), 6.66 (2H, s, H-2, 6), 5.50 (1H, d, $J = 8.0$ Hz, H-7), 3.78 (2H, m, H-9), 3.57 (2H, t, $J = 6.5$ Hz, H-9'), 3.46 (1H, m, H-8), 2.63 (2H, t, $J = 7.5$ Hz, H-7'), 1.81 (2H, m, H-8'), 3.79 (6H, s, -OCH₃), 3.85 (3H, -OCH₃); ¹³C-NMR (100 MHz, CD₃OD): δ 149.3 (C-3, 5), 147.5 (C-4'), 145.2 (C-3'), 136.9 (C-4, 1'), 134.0 (C-5'), 129.8 (C-1), 117.9 (C-6'), 114.1 (C-2'), 104.1 (C-2, 6), 89.1 (C-7), 65.0 (C-9), 62.2 (C-9'), 56.78 (3'-OCH₃), 56.75 (3, 5-OCH₃), 55.5 (C-8), 35.8 (C-8'), 32.9 (C-7').

5-Methoxydihydrodehydroconiferyl alcohol (33)

White amorphous powder; $[\alpha]_{D}^{21} -1.6^{\circ}$ ($c=0.36$, MeOH), ¹H-NMR (400 MHz, CD₃OD): δ 6.94 (1H, d, $J = 1.70$ Hz, H-2), 6.82 (1H, dd, $J = 8.05, 1.70$ Hz, H-6), 6.74 (1H, d, $J = 8.05$ Hz, H-5), 6.71 (2H, s, H-2', 6'), 5.47 (1H, d, $J = 6.10$ Hz, H-7), 3.84 (3H, s, -OCH₃), 3.80 (3H, s, -OCH₃), 3.74 (2H, m, H-9), 3.55 (2H, t, $J = 6.5$ Hz, H-9'), 3.44 (1H, m, H-8), 2.61 (2H, t, $J = 7.3$ Hz, H-7'), 1.80 (2H, m, H-8'); ¹³C-NMR (100 MHz, CD₃OD): δ 149.0 (C-3), 147.5 (C-4), 147.4 (C-4'), 145.2 (C-3'), 136.9 (C-1'), 134.8 (C-5'), 129.8 (C-1), 119.7 (C-6), 117.9 (C-6'), 116.1 (C-5), 114.1 (C-2'), 110.5 (C-2), 88.9 (C-7), 64.9 (C-9), 62.2 (C-9'), 56.7 (-OCH₃), 56.3 (-OCH₃), 55.4 (C-8), 35.8 (C-8'), 32.9 (C-7').

Spirosta-5,25(27)-diene-1 β ,3 β -diol (neoruscogenin) 1-O-[O- α -L-rhamnopyranosyl]- (1,2)- α -L-arabinopyranoside] (34)

White amorphous powder; FAB-MS m/z : 707 $[M+H]^+$, 729 $[M+Na]^+$; 1H -NMR (500 MHz, pyridine- d_5): δ 6.35 (1H, s, H-Rha-1), 5.56 (1H, d, $J = 5.5$ Hz, H-6), 4.82 (1H, m, H-Rha-5), 4.78 (1H, s, H-27), 4.74 (1H, s, H-27), 4.71 (1H, d, $J = 2.9$ Hz, H-Rha-2), 4.70 (1H, d, $J = 7.3$ Hz, H-Ara-1), 4.68 (1H, dd, $J = 9.4, 2.9$ Hz, H-Rha-3), 4.62 (1H, dd, $J = 8.4, 7.4$ Hz, H-Ara-2), 4.57 (1H, m, H-16), 4.44 (2H, d, $J = 12.0$ Hz, H-26), 4.29 (1H, dd, $J = 9.4, 9.4$ Hz, H-Rha-4), 4.25 (1H, dd, $J = 12.0, 2.2$ Hz, H-Ara-5a), 4.13 (2H, overlap, H-Ara-3, 4), 3.82 (1H, m, H-3), 3.80 (1H, m, H-1), 3.65 (1H, d, $J = 12.0$ Hz, H-Ara-5b), 1.71 (3H, d, $J = 6.2$ Hz, H-Rha-6), 1.42 (3H, s, CH₃-19), 1.03 (3H, d, $J = 6.9$ Hz, CH₃-21), 0.83 (3H, s, CH₃-18); ^{13}C -NMR (125 MHz, pyridine- d_5): δ 83.5 (C-1), 37.4 (C-2), 68.1 (C-3), 43.8 (C-4), 139.7 (C-5), 124.7 (C-6), 31.9 (C-7), 33.1 (C-8), 50.3 (C-9), 42.8 (C-10), 24.0 (C-11), 40.3 (C-12), 40.3 (C-13), 56.7 (C-14), 32.3 (C-15), 81.4 (C-16), 62.9 (C-17), 16.7 (C-18), 15.1 (C-19), 41.8 (C-20), 15.0 (C-21), 109.4 (C-22), 33.2 (C-23), 28.9 (C-24), 144.6 (C-25), 65.0 (C-26), 108.6 (C-27), 100.4 (C-Ara-1), 75.0 (C-Ara-2), 75.9 (C-Ara-3), 70.1 (C-Ara-4), 67.3 (C-Ara-5), 101.6 (C-Rha-1), 72.5 (C-Rha-2), 72.6 (C-Rha-3), 74.2 (C-Rha-4), 69.4 (C-Rha-5), 19.0 (C-Rha-6).

(25R)-Spirost-5-en-3-ol-3-O- α -L-rhamnopyranosyl-(1,2)-[β -D-glucopyranosyl-(1,3)]- β -D-glucopyranoside (35)

White amorphous powder; 1H -NMR (400 MHz, pyridine- d_5): δ 6.31 (1H, brs, H-Rha-1), 5.30 (1H, brs, H-6), 5.04 (1H, m, H-Glc-1'), 4.87 (1H, m, H-Glc-1), 1.75 (3H, d, $J = 6.0$ Hz, H-Rha-6), 1.13 (3H, d, $J = 6.7$ Hz, CH₃-21), 1.04 (3H, s, CH₃-19), 0.80 (3H, s, CH₃-18), 0.66 (3H, s, CH₃-27); ^{13}C -NMR (100 MHz, pyridine- d_5): δ 37.1 (C-1), 30.0 (C-2), 78.3 (C-3), 38.6 (C-4), 140.7 (C-5), 121.8 (C-6), 32.1 (C-7), 31.8 (C-8), 50.2 (C-9), 37.4 (C-10), 21.0 (C-11), 39.8 (C-12), 40.4 (C-13), 56.6 (C-14), 32.2 (C-15), 81.0 (C-16), 62.8 (C-17), 16.3 (C-18), 19.3 (C-19), 41.9 (C-20), 15.0 (C-21), 109.2 (C-22), 30.0 (C-23), 29.2 (C-24), 30.5 (C-25), 66.8 (C-26), 17.2 (C-27), 99.9 (C-Glc-1), 78.3 (C-Glc-2), 88.1 (C-Glc-3), 77.0 (C-Glc-4), 78.5 (C-Glc-5), 62.8

(C-Glc-6), 102.3 (C-Rha-1), 72.4 (C-Rha-2), 72.8 (C-Rha-3), 74.0 (C-Rha-4), 69.6 (C-Rha-5), 18.6 (C-Rha-6), 105.4 (C-Glc-1'), 75.0 (C-Glc-2'), 77.6 (C-Glc-3'), 72.4 (C-Glc-4'), 77.9 (C-Glc-5'), 62.8 (C-Glc-6').

Compound 36

Yellow amorphous powder; ESI-MS m/z : 667 [2M-H]⁻, 333 [M-H]⁻, 255 [M-HSCH₂CH₂OH-H]⁻; ¹H-NMR (CD₃OD, 400 MHz) δ : 7.11 (2H, d, J = 8.5 Hz, H-2' and H-6'), 6.78 (1H, d, J = 8.0 Hz, H-6), 6.73 (2H, d, J = 8.5 Hz, H-3' and H-5'), 6.35 (1H, d, J = 2.5 Hz, H-3), 6.26 (1H, dd, J = 2.5, 8.5 Hz, H-5), 3.71 (3H, s, OCH₃), 3.68 (1H, m, H- γ), 3.46 (2H, t, J = 6.5 Hz, -CH₂OH), 2.40 (4H, m, H- α , -CH₂S), 2.00 (2H, m, H- β). ¹³C-NMR (CD₃OD, 100 MHz) δ : 159.7 (C-2), 157.9 (C-4), 157.4 (C-4'), 134.7 (C-1'), 131.2 (C-6), 130.2 (C-2', C-6'), 121.9 (C-1), 116.1 (C-3', C-5'), 107.5 (C-5), 99.8 (C-3), 62.3 (-CH₂OH), 55.6 (-OCH₃), 49.7 (C- γ), 38.0 (C- β), 33.9 (-CH₂S), 28.9 (C- α).

Compound 37

Yellow amorphous powder; ESI-MS m/z : 787 [2M-H]⁻, 393 [M-H]⁻, 255 [M-HSCH₂CH₂SCH₂CH₂OH-H]⁻; ¹H-NMR (CD₃OD, 400 MHz) δ : 7.09 (2H, d, J = 8.4 Hz, H-2' and H-6'), 6.76 (1H, d, J = 8.4 Hz, H-6), 6.71 (2H, d, J = 8.4 Hz, H-3' and H-5'), 6.32 (1H, d, J = 2.0 Hz, H-3), 6.23 (1H, dd, J = 2.4, 8.0 Hz, H-5), 3.70 (3H, s, OCH₃), 3.66 (1H, m, H- γ), 3.50 (2H, t, J = 6.4 Hz, -CH₂OH), 2.45 (8H, m, H- α , -CH₂S), 1.95 (2H, m, H- β). ¹³C-NMR (CD₃OD, 100 MHz) δ : 159.6 (C-2), 157.9 (C-4), 157.4 (C-4'), 134.5 (C-1'), 131.2 (C-6), 130.2 (C-2', C-6'), 121.7 (C-1), 116.1 (C-3', C-5'), 107.5 (C-5), 99.7 (C-3), 62.3 (-CH₂OH), 55.5 (-OCH₃), 49.6 (C- γ), 37.8 (C- β), 35.1, 33.1, 32.0 (-CH₂S), 28.8 (C- α).

Compound 38

Yellow amorphous powder; ESI-MS m/z : 907 [2M-H]⁻, 453 [M-H]⁻, 255

[M-HSCH₂CH₂SCH₂CH₂SCH₂CH₂OH-H]⁻; ¹H-NMR (CD₃OD, 400 MHz) δ: 7.13 (2H, d, *J* = 8.4 Hz, H-2' and H-6'), 6.78 (1H, d, *J* = 8.0 Hz, H-6), 6.74 (2H, d, *J* = 8.4 Hz, H-3' and H-5'), 6.36 (1H, d, *J* = 2.4 Hz, H-3), 6.27 (1H, dd, *J* = 2.4, 8.0 Hz, H-5), 3.72 (3H, s, OCH₃), 3.69 (1H, m, H-γ), 3.65 (2H, t, *J* = 6.8 Hz, -CH₂OH), 2.54 (12H, m, H-α, -CH₂S), 1.97 (2H, m, H-β). ¹³C-NMR (CD₃OD, 100 MHz) δ: 159.6 (C-2), 157.9 (C-4), 157.5 (C-4'), 134.5 (C-1'), 131.3 (C-6), 130.2 (C-2', C-6'), 121.7 (C-1), 116.2 (C-3', C-5'), 107.5 (C-5), 99.8 (C-3), 62.5 (-CH₂OH), 55.6 (-OCH₃), 49.6 (C-γ), 37.8 (C-β), 35.3, 33.3, 33.1, 33.0, 32.0 (-SCH₂), 28.9 (C-α).

Compound 39

Yellow amorphous powder; FAB-MS *m/z*: 367 [M+H]⁺, ¹H-NMR (CD₃OD, 400 MHz) δ: 7.01 (2H, d, *J* = 8.4 Hz, H-2' and H-6'), 6.61 (2H, d, *J* = 8.4 Hz, H-3' and H-5'), 5.94 (2H, dd, *J* = 7.2, 1.6 Hz, H-2 and H-6), 5.92 (1H, *J* = 1.6 Hz, H-4), 3.94 (1H, t, *J* = 8.4 Hz, -CH), 3.46 (2H, t, *J* = 6.4 Hz, -CH₂OH), 2.77 (2H, m, -CH₂), 2.43 (6H, m, -CH₂S). ¹³C-NMR (CD₃OD, 100 MHz) δ: 159.1 (C-3, C-5), 157.5 (C-4'), 142.7 (C-1), 134.1 (C-1'), 130.2 (C-2', C-6'), 116.1 (C-3', C-5'), 108.8 (C-2, C-6), 101.5 (C-4), 62.4 (-CH₂OH), 51.9 (-CH), 44.5 (-CH₂), 35.2, 33.0, 32.4 (-SCH₂).

Pinosylvin (40)

White amorphous powder; ¹H-NMR (CD₃OD, 400 MHz) δ: 7.50 (2H, d, *J* = 7.6 Hz, H-2' and H-6'), 7.33 (2H, t, *J* = 7.3 Hz, H-3' and H-5'), 7.21 (1H, t, *J* = 7.3 Hz, H-4'), 7.05 (1H, d, *J* = 16.3 Hz, H-α), 6.99 (1H, d, *J* = 16.3 Hz, H-β), 6.48 (2H, d, *J* = 2.2 Hz, H-2 and H-6), 6.19 (1H, t, *J* = 2.2 Hz, H-4).

Methylation of the polymer fraction

Polymeric polyphenols (Fraction 5, 100 mg) was dissolved in MeOH (5 mL) and treated with ethereal CH₂N₂ at 0 °C for 12 h. The mixture was concentrated by rotary evaporator and analyzed by ESI-MS.

Chapter 4

Separation of polymeric polyphenols

Cinnamon tea (50 g) was extracted with 60% acetone for 3 times at room temperature. The extract was concentrated under reduced pressure until the organic solvent was removed and the resulting aqueous solution was partitioned with AcOEt. A portion of the AcOEt layer (1.0 g/5.9 g) was subjected to size-exclusion chromatography using Sephadex LH-20 column (2 cm i.d. × 45 cm) with 7 M urea-acetone (4:6, v/v) as eluent to give two fractions. Fraction A containing polymeric substance was applied to Diaion HP 20SS (2 cm i.d. × 18 cm, 30% ~ 100% MeOH) to yield polymeric polyphenols (22.4 mg). The precipitate (1.0 g/3.9 g) formed during solvent partitioning was separated by the same size-exclusion chromatography to give polymer fractions, C-1 (23.5 mg), C-2 (164.3 mg), C-3 (132.5 mg), and C-4 (68.3 mg). The ¹³C NMR experiment indicated Fr. C-3 was polymeric polyphenols originated from black tea used for production of the cinnamon tea.

Reaction of tea catechin with cinnamaldehyde

(-)-Epigallocatechin-3-*O*-gallate (**41**, 10 g) and cinnamaldehyde (**42**, 2.9 g) were dissolved in EtOH (1% TFA, 40 mL) and kept at room temperature for 2 days. The mixture was separated into 7 fractions by Sephadex LH-20 column chromatography (4 cm i.d. × 23 cm) with EtOH/H₂O/acetone (0.1% TFA). Fraction 7 (1.0 g/11.8 g) was successively subjected to column chromatography using silica gel (CHCl₃/MeOH/ H₂O, 90:10:1 ~ 60:40:10) and ODS (20% ~ 100% MeOH, 10% stepwise elution) to yield **43** (10.5 mg). The major products of this reaction were polymeric substance.

Compound 43

Tan amorphous powder; $[\alpha]_D^{26}$ -131.07° (c=0.12, MeOH); ESI-MS: *m/z* 1167.2

[M+Na]⁺, HR-FAB-MS: m/z 1167.2543 [M+Na]⁺, (Calcd for C₆₂H₄₈O₂₂Na: 1167.2536); IR cm⁻¹: 3732, 3626, 1696, 1539, 668,; UV λ_{\max} (MeOH) nm (log ϵ): 268 (3.23).

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